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# THE JOURNAL OF HYGIENE

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## ON A COMPLEMENT-STIMULATING SUBSTANCE IN COW'S MILK.

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King's College.)*

IN a former communication (1) we showed that a substance acting as complement is present in milk under both normal and abnormal conditions, and that this substance displays characteristics which lend a certain amount of doubt as to its true complementary nature. Expression was also given to the idea, that the haemolytic system—heated ox-serum + guinea-pig corpuscles—is of a peculiarly sensitive character in regard to ox-complement in the presence of milk.

We have made a considerable number of experiments directed towards the elucidation of these points, but whilst an answer to the former has not been satisfactorily obtained the latter has been well substantiated.

### THE NATURE OF THE COMPLEMENTARY SUBSTANCE IN MILK.

#### (a) *The effect of heating to 55° C. for 30 minutes.*

In all cases this was effective in destroying the complement activity of milk.

This however is also stated to be true of certain pseudo-complementary substances such as oleates, in presence of protein.

(b) *The effect of filtration through porous porcelain, under pressure.*

The complementary substance of milk does not appear to pass such filters, the whey obtained being quite inactive. A similar result is obtained when ox-complement is added to milk before filtration.

(c) *The effect of the removal of calcium salts on the complement activity of milk.*

It is usually held that the presence of calcium salts has an inhibitory effect on complement activity. As milk contains a considerable percentage of calcium salts, experiments were made with a milk, which ordinarily showed strong haemolytic activity, both before and after removal of calcium by the addition of sodium oxalate.

If 0.4 per cent. of solid sodium oxalate be added to milk and the mixture allowed to stand for a short time and then centrifuged, practically the whole of the calcium in the milk is replaced by sodium, and calcium oxalate is precipitated. At the same time the milk undergoes a decided change in reaction; for instance a milk showing 15 degrees of acidity (*i.e.* 100 c.c. of milk require 15 c.c. of N/10 NaOH to produce a pink colour with phenol-phthalein) after treatment with sodium oxalate only shows an acidity of about 4–5 degrees. Milk so treated with sodium oxalate shows an identical haemolytic action before and after treatment even when no attempt was made to restore the original hydrogen-ion concentration. When this was done, the haemolytic activity of the treated milk appeared to be somewhat less than that of the untreated milk.

(d) *The effect of shaking.*

Many investigators hold that complement is destroyed fairly readily by agitation of a more or less severe character. A great number of experiments were made with (1) milk which possessed naturally a strong haemolytic activity, (2) milk, inactivated at 55°, to which an addition of ox-complement had been made, (3) saline with a similar addition of ox-complement. These mixtures were placed, in quantities of 20 c.c., in strong glass test tubes (12.5 cm.  $\times$  2.5 cm.) and shaken in a Hearson shaker, both with and without the addition of glass beads. The shaking was very vigorous (about 250 vibrations per minute) and

tests of the haemolytic activity were made after intervals of 15, 30, 45 and 60 minutes. The temperature of shaking was that of the room (*circa* 65°–70° F.). These experiments led to very little information of a direct character, as the greatest difficulty was experienced in bringing about any appreciable destruction of complement. There was, perhaps, very slight evidence of such destruction but not of sufficient magnitude to throw any light on the character of the substance which naturally occurs in milk.

During the course of these experiments, however, it was noticed that, whereas the concentration of ox-complement in the saline and in the inactivated milk was the same in any individual experiment, yet, on testing the two series for haemolytic activity, the haemolysis in the milk series was relatively very much more powerful than in the corresponding saline series, and in fact the difference was of a very marked character. This observation led to a careful investigation into a possible stimulating effect of milk on complement.

#### THE PRESENCE OF A SPECIFIC COMPLEMENT-STIMULATING SUBSTANCE IN MILK.

Determinations of the limits of haemolytic activity were made with the system—heated ox-serum 0.3 c.c. + guinea-pig corpuscles (1:20) 0.5 c.c. + diluted complement 1 c.c.—using various substances as the complement diluent and also complement from various sources.

The following signs are used to signify the amount of haemolysis obtained:

+	+	+	+	=complete haemolysis.
+	+	+		=nearly complete, but slight deposit of unlaked cells.
+	+			} =partial haemolysis, with increasing deposit of cells.
+				
sl. +				
—				=no haemolysis.

It will only be necessary to state once and for all that all the required controls were made in conjunction with the following experiments. No experiment is recorded in which there was any failure in this respect.

The dilutions employed were made with the utmost care, as far as possible the same pipettes being employed in each series.

*Complement-stimulating Substance*

EXP. 1. *Diluents.* Saline and inactivated cow's milk.

*Complement.* Ox.

Dilutions	Haemolysis in	
	Saline	Milk
1: 20	+++	++++
1: 50	+++	++++
1: 200	—	+++

EXP. 2. *Diluents.* Saline and two different samples of inactivated cow's milk, both of which gave reactions for the presence of complementary substance before inactivation.

*Complement.* Ox.

Dilutions	Haemolysis in		
	Saline	Milk 1	Milk 2
1: 25	++++	++++	++++
1: 50	++	++++	++++
1: 100	sl. +	++++	+++
1: 150	—	+++	++
1: 200	—	++	+
1: 250	—	+	+
1: 300	—	+	sl. +
1: 400	—	sl. +	v.sl. +
1: 500	—	—	—

The haemolysis obtained in 1 c.c. of each of these milks before inactivation was (1) ++; (2) +++. It is to be noted that the latter had a somewhat slighter stimulating action.

EXP. 3. As Exp. 2 but using as diluents saline and a farmer's mixed milk, inactivated and unactivated.

Dilutions	Haemolysis in		
	Saline	Inactivated Milk	Unactivated Milk
1: 25	++++	++++	} identical with the inactivated milk series
1: 50	++++	++++	
1: 100	? v.sl. +	++++	
1: 150	—	+++	
1: 200	—	+++	
1: 250	—	++	
1: 300	—	++	
1: 400	—	+	
1: 500	—	+	

From the above three experiments, the stimulating action of milk on ox-complement is easily seen, and the inactivation of the milk by heat at 55° C. neither contributes nor detracts from the effect. This

stimulating effect was always obtained though in varying degrees of intensity. The thermostability of the stimulating substance was next ascertained.

EXP. 4. *Diluents.* Inactivated cow's milk and the same milk heated in boiling water for 10 minutes.

*Complement.* Ox.

Dilutions	Haemolysis in	
	Boiled Milk	Inactivated Milk
1: 25	+++	++++
1: 50	+	++++
1: 100	—	+++
1: 150	—	++
1: 200	—	+
1: 250	—	+
1: 300	—	sl. +
1: 400	—	v.sl. +
1: 500	—	—

The heating of milk to the temperature of boiling water destroys or changes the substance which produces the stimulating effect. The same experiment was carried out in order to determine as nearly as possible at what temperature the destruction of this substance occurs. For this purpose milk was heated (1) at 55° C. for 30 minutes, (2) at 67–68° C. for 10 minutes, (3) at 72–73° C. for 10 minutes, and (4) in boiling water for seven minutes.

The results were as follows:

Dilutions	Haemolysis in				
	Saline	Milk 55°	Milk 67°	Milk 72°	Milk 100°
1: 10	++++	++++	++++	++++	++++
1: 25	+++	+++	+++	+++	++
1: 50	+	++	+	+	sl. +
1: 75	sl. +	+	sl. +	v.sl. +	v.sl. +
1: 100	—	sl. +	—	—	—
1: 150	—	v.sl. +	—	—	—

This series did not show the marked differences which were sometimes obtained, but it is not easy to express on paper differences which are easily detected by the eye. There was however distinct inhibition by the boiled milk, distinct stimulation by the milk heated at 55°, slight inhibition by the milk heated at 72°, and practically no effect in either direction by the milk heated at 67°. It would appear that the destruction of this stimulating substance begins in the neighbourhood of that temperature at which albumin coagulation occurs, but as will be seen (Exp. 5) the lact-albumin does not appear to be concerned in the stimulating effect.



## Complement-stimulating Substance

EXP. 5. The filtration of cow's milk through Doulton filters in order to see whether the stimulating substance would pass with the whey.

*Diluents.* In all cases: saline, inactivated cow's milk, and inactivated filtered whey of same milk.

*Complement.* In all cases: ox.

(1)	Dilutions	Haemolysis in		
		Saline	Milk	Whey
	1: 50	+++	++++	++++
	1: 100	—	++++	+++
	1: 200	—	? v.sl. +	—
	1: 300	—	—	—
	1: 400	—	—	—

In this case the stimulating substance appeared to pass the filter.

(2)	Dilutions	Haemolysis in		
		Saline	Milk	Whey
	1: 25	+	+++	++
	1: 50	?	++	?
	1: 100	—	sl. +	—
	1: 200	—	—	—

The whey had a slight stimulating influence, but was not so energetic as the milk itself.

(3)	Dilutions	Haemolysis in			Whey heated to 75° C. for 5 minutes and filtered
		Saline	Milk	Whey	
	1: 10	++++	++++	++++	++++
	1: 25	+++	+++	+++	+++
	1: 50	? v.sl. +	+	? v.sl. +	sl. +
	1: 75	—	sl. +	—	—
	1: 100	—	? v.sl. +	—	—
	1: 150	—	—	—	—
	1: 200	—	—	—	—

The whey showed a slight stimulating power over saline and the heated whey had a slightly more powerful action, but neither had the same effect as the milk itself.

(4)	Dilutions	Haemolysis in		
		Saline	Milk	Whey
	1: 25	++	++++	+++
	1: 50	v.sl. +	++	sl. +
	1: 100	—	sl. +	—
	1: 150	—	—	—
	1: 200	—	—	—

As in the other experiments the whey appears to have a slight stimulating effect as compared with the saline, but is not so active as the milk itself.

As a result of these experiments, it is evident that the greater part of the stimulating substance remains with the casein on the filter, or else is destroyed or altered during its passage.

Exp. 6. Similar to Exp. 4 but using guinea-pig complement in the place of ox-complement.

*Diluents.* Saline and

Cow's milk (a) Heated in boiling water 5 minutes.

.. (b) .. at 72° C., 10 minutes.

.. (c) .. at 55° C., 30 minutes.

*Complement.* Guinea-pig.

Dilutions	Haemolysis in			
	Saline	(a)	(b)	(c)
1: 10	++++	v.sl. +	++++	++++
1: 25	++++	—	+++	+++
1: 50	+++	—	++	++
1: 75	++	—	+	+
1: 100	+	—	v.sl. +	sl. +
1: 150	+	—	—	v.sl. +
1: 200	v.sl. +	—	—	—
1: 250	—	—	—	—

These results are remarkable. Cow's milk heated at 55° C. for 30 minutes exerts *no* stimulating effect on guinea-pig complement, in fact there is inhibition (probably due to the calcium salts present). Heating at 72° C. renders cow's milk a little more inhibitory and heating in boiling water renders the milk almost completely inhibitory to guinea-pig complement.

The distinction between these two forms of complement is shown again in the following experiment.

The guinea-pig complement was diluted 1:1 with saline before making the further dilutions with saline and milk.

Dilutions	Ox-complement Haemolysis		G.P. complement Haemolysis	
	Saline	Milk	Saline	Milk
1: 10	++++	++++	++	sl. +
1: 25	++	++++	sl. +	—
1: 50	? v.sl. +	++++	—	—
1: 75	—	+++	—	—
1: 100	—	++	—	—
1: 150	—	sl. +	—	—
1: 200	—	—	—	—

The stimulating action of cow's milk on ox-complement and its inhibitory action on guinea-pig complement is easily seen.

The next step was to ascertain whether the same stimulating effect was produced on ox-complement, by milk from heterogeneous sources.

*Complement-stimulating Substance*

EXP. 7. *Diluents.* Saline, inactivated cow's milk and inactivated asses' milk.

*Complement.* Ox.

Dilutions	Haemolysis in		
	Saline	Cow's Milk	Asses' Milk
1: 10	+++	+++	+++
1: 25	++	+++	+++
1: 50	v.sl. +	+++	v.sl. +
1: 75	—	+++	?
1: 100	—	++	—
1: 150	—	sl. +	—
1: 200	—	—	—

Asses' milk has apparently neither stimulating nor inhibitory action on ox-complement, the saline and asses' milk series being practically identical.

The experiment was then varied by using other sources of complement both with cow's milk and also with the homologous milk.

EXP. 8. *Diluents.* Saline, inactivated cow's milk and inactivated asses' milk.

*Complement.* Ass.

Dilutions	Haemolysis in		
	Saline	Cow's Milk	Asses' Milk
1: 10	+++	+++	+++
1: 20	+	+	+
1: 25	v.sl. +	v.sl. +	v.sl. +
1: 33	—	—	—
1: 50	—	—	—
1: 65	—	—	—
1: 100	—	—	—

It is evident that asses' milk does not stimulate asses' complement in the same way that cow's milk stimulates ox-complement.

EXP. 9. *Diluents.* Saline, inactivated cow's milk and inactivated human milk.

*Complement.* Human.

Dilutions	Haemolysis in		
	Saline	Cow's Milk	Human Milk
1: 10	+++	+++	++
1: 25	+++	+	—
1: 50	++	—	—
1: 75	+	—	—
1: 100	sl. +	—	—
1: 150	—	—	—

In this case both the milks have a strong inhibitory action on human complement, the action being greater in the case of the human milk. This can scarcely be due to calcium salts as probably there is a smaller percentage of these in the human milk than in the cow's milk.

As the result of these experiments, it was evident that the stimulating effect is only exerted in an entirely homologous system. An experiment was therefore made, using a parallel system, in order to see if the same result would be obtained, as had been, in the case of cow's milk.

The parallel system was: sheep's corpuscles (1:20) 0.5 c.c. + inactivated human serum 0.5 c.c. + human-complement + human milk. This is strictly analogous to the original system: guinea-pig corpuscles + heated ox-serum + ox-complement + cow's milk. Three parallel tests were made using as diluents (1) human milk, (2) saline, (3) cow's milk.

The results were as follows:

Dilutions	Haemolysis in		
	Saline	Cow's Milk	Human Milk
1: 10	++	v.sl. +	—
1: 25	v.sl. +	—	—
1: 50	—	—	—
1: 75	—	—	—
1: 100	—	—	—
1: 125	—	—	—

The result is quite the reverse of the original system, and it is necessary to suppose that the stimulating action is specific for the original system only.

It was necessary however to ascertain whether the stimulating effect was directed towards the complement, or whether it was the amboceptor that was affected. In order to ascertain this, sheep's corpuscles and horse-serum rendered haemolytic to these corpuscles were used. The system was: 0.4 c.c. horse-serum (100 times the minimum haemolytic dose) + 0.5 c.c. sheep's corpuscles (1:20) + 1 c.c. of the dilution of ox-complement. The diluents were saline and inactivated cow's milk. The results were as follows:

Dilutions	Haemolysis in	
	Saline	Cow's Milk
1: 5	+	+
1: 10	sl. +	sl. +
1: 20	v.sl. +	v.sl. +
1: 50	—	—
1: 75	—	—
1: 100	—	—

The haemolysis was identical in each case whilst a parallel experiment using the original heated ox-serum and guinea-pig corpuscles system showed the usual stimulating effect. The use of the above system in which sheep's corpuscles play a part is rendered somewhat difficult on account of the agglutination of the corpuscles which occurs. Further, it has been noticed constantly that towards the delicate system—sheep's corpuscles + the corresponding horse-serum amboceptor—ox-serum appears to contain but little complement. For instance, whereas with this system complete haemolysis occurred in a few minutes with 1:10 guinea-pig complement, haemolysis was not complete in two hours, even when undiluted ox-complement was employed, though possibly the agglutination of the sheep's corpuscles played some part in this inhibition.

The exhibition of this stimulating effect of milk on haemolytic action is to a certain extent dependent on the quantity of inactivated ox-serum used as amboceptor. The divergence between the results in saline and milk is most marked when the least possible quantity of amboceptor is used. If excessive amounts of ox-serum amboceptor be used, the result may be reversed.

The results recorded above appear to admit of little explanation, though they explain to a certain extent some of the divergent views which have been published as to the presence of complement in milk.

The following view has been expressed by Liebermann and V. Fenyvissy.

“One can attain, by the use of mixtures of sodium soaps with various substances present in normal sera which contain natural complement, much stronger haemolysis of sensitised corpuscles than normal. As particularly useful in this connection may be noted, combinations which besides soap, contain serum globulin and calcium chloride and an alkalinity suitable to the combination.” How very closely milk corresponds to this necessary combination is obvious. At the same time, there seems no reason why the exhibition of this stimulating power should be confined to cow's milk and to one particular haemolytic system.

#### REFERENCE.

- (1) HEWLETT, R. TANNER and REVIS, C. (1914). On the presence of so-called “Complement” in Milk. *Journal of Hygiene*, xiv. 481–497.



# STUDIES IN THE MEANING AND RELATIONSHIPS OF BIRTH AND DEATH RATES.

## II.

*Density of Population and Death Rate (Farr's Law).*

By JOHN BROWNLEE, M.D., D.Sc.,

*Statistician, Medical Research Committee.*

THIS subject was first considered statistically by the late Dr Farr. It is one of the brilliant attempts to extract the real meaning of figures so frequent in his work, but though this theory has not shared in the complete neglect that has been the lot of his attempt to put a quantitative measure to the course of epidemics, it has suffered as much from the kind of patronage with which it is usually discussed. On at least one of the great medical officers of health of his time, however, — the late Dr J. B. Russell—the theory exercised a strong fascination. My own copy of Farr's *Vital Statistics* came from Dr Russell's library, and the whole passage referring to the law is lined with his characteristic nervous pencil marks, while in much of his work on vital statistics the influence can easily be traced.

The neglect of the subject is of two-fold origin. In the first place the law appeared quite artificial. In the second place the statistics of the decade on which it was founded happened to be specially suitable for its discovery, while subsequent figures did not appear to afford the same support.

The law itself, if the death rate be denoted by  $R$  and the density of population (say the number of persons per square mile) by  $D$ , is that

$$R = cD^m,$$

when  $c$  and  $m$  are constants.

But how is the death rate to be measured? By Farr the crude death rate was used and found to give a good measure of the facts.

Later when corrected death rates were substituted, and that seemed to be the proper course, the law obviously did not hold, and even with crude death rates, its success as a descriptive formula was not nearly so marked. Thus in the absence of any *a priori* justification the law was relegated to a somewhat obscure position. Before proceeding to its justification, however, it is necessary to have a clear idea of what kind of evidence can be produced. The law must be a law of average, for on account of the arbitrary nature of the boundaries of the registration districts, the number of persons living on an acre is merely a rough approximation. The groups of localities which supply the figures must further be large, as some with better conditions will have lower death rates, and others with worse, a higher. Nor can even a large city be divided into small districts and these considered. A city population must be a whole population; the slum is not wholly recruited from the slum by any means. A district consisting chiefly of persons engaged in trades and minor occupations may have a very high density and yet a low death rate. All, or at least the great majority of the inhabitants are respectable, those who are not, are driven elsewhere, yet the latter must be considered as part of the same population: from this class, also, though some ascend in the social scale, they do not constitute a separate population. It is obvious therefore that to obtain a suitable average a few groups only must be chosen. Dr Farr made seven, Dr Tatham sixteen; the former may be too few, the latter seems too many. The effect of density is not merely as density. The country preserves life even in the presence of excess or dissipation: the town does not. Further, in the period of growth, children in the city do not get anything like the same chance as their fellows in the country, even though housing may be better and food more abundant. In addition filth in the country is at its worst in most cases but a local nuisance, spreading enteric fever and diarrhoea at times, but not having the power of rendering a whole district foetid. All these influences act concurrently and cumulatively to depress health the more closely people are crowded together, and as life is a physico-chemical process this effect must be measurable and should be capable of expression in some formula which goes back to chemistry and physics. Such a formula is that of Dr Farr. Nothing comparable to it was known in his day, so that as a mathematical formula can easily be found to describe almost any statistics, his formula seemed just such an one and no better than many others. It is, however, no longer alone.

This subject I investigated many years ago without making any

advance. The difficulty of defining a death rate was too great. In my last paper, however, I have given a method for obtaining the death rate on a stationary population, and the application of this method justifies the law.

In order to illustrate the subject as fully as possible, two tables have been constructed, one showing the figures used by Dr Farr which refer to the decade 1861-1870, the second the comparative table for the decade 1891-1900 as given by Dr Tatham. Dr Farr used the crude death rate. Fortunately, he has also published the death rates at each age period for the groups of populations on which he based his law. This allows death rates to be calculated on the same standard population which has been used in framing the figures of the second table, and from these life table death rates, which are strictly comparable with those in the second table, have been calculated. The constants of the curves of the form  $R = cD^m$  have been evaluated by the method of least squares for both periods, for the crude death rates, the corrected death rates, and the life table death rates.

It will be noticed that the values of  $m$  roughly correspond for each separate case in two periods, but in the case of the life table death rates, they correspond within three places of decimals, the furthest that could be statistically expected. We thus have a quite definite law acting independently of the changes which have taken place due to sanitary progress. Improve all round and the exponent does not vary, but only the multiplying constant. The former constant  $m$  therefore represents the law, and the latter  $c$  may be called the co-efficient of intensity of unhealthiness in the country. This co-efficient  $c$  will vary as sanitary conditions improve or the reverse, though the law will remain the same.

When the columns showing the results obtained by fitting similar curves to the crude and corrected death rates are compared, it is seen that the crude death rate fits less well than the life table death rate and that the corrected death rates are very badly represented by the formula. This is what would be expected from the fact shown in the previous paper that life table death rate can be obtained by multiplying the corrected death rate by one constant and adding a second. It will be noticed that the crude death rate curve of Dr Farr has an exponent of  $\cdot1193$  which is much nearer the probable true exponent  $\cdot100$  than that of the crude death rate for the decade 1891-1900 which is  $\cdot1276$ . This is explained by the fact that in the earlier period the crude death rate was 22.42 as against a life table death rate of 24.06, while in the latter

period the corresponding figures are 18.19 and 21.77. Dr Farr had thus a better opportunity of formulating a law than his successors. With the crude death rate diverging more and more from the life table death rate it became more and more difficult to accept the relationship demanded by the formula. A law of which the main feature, namely, the exponent  $m$ , varied could hardly lay claim to be a law at all, any more than the law of gravitation could be justified if the relationship were not constantly the inverse square. Using a death rate which is more comparable between populations, namely, that which would hold if the population were stationary, the exponent takes the same value. It will be noticed that the co-efficient  $c$  decreases from 12.42 in the first period to 10.83 in the latter. In other words, density has only .875 times the effect in producing mortality it had in 1860-1870, so much have sanitary conditions improved.

But the law remains apparently. Sanitation may diminish  $c$ , but the ill effects of concentration do not seem capable of being changed merely by sanitation. What the figures just given clearly mean is, that on the whole, conditions of life in modern England seem to be so uniformly the result of the action of the modern developments of industrialism, etc., as to be comprehended in a formula. The prospect that the town may become as healthy as the country, given proper precautions of living, does not seem possible if any law like that of Farr is found to hold permanently. In any case, decrease of density is essential.

But there is one exception to this law in both periods, namely, that of London. In 1861-70, the life table death rate of London was 26 per mille as against 32 expected by the formula. Unless this can be explained the formula falls. But I think it can be explained. Modern England was in 1860, and still is, a recent phenomenon compared with London. Liverpool, Manchester, etc., are but mushroom growths of yesterday. London began to pay its 'prentice fee' as a city in the middle of the seventeenth century. More than a century ago it had a million inhabitants. Sanitation was unknown. Countless thousands tried to live in it and failed. It was in the contemporary documents the 'wen' or the 'vampyre' that sucked England's blood. It was fifty years later than the rest of England in having a birth rate in excess of its death rate, and now it has its reward, the result of two centuries of natural selection in its crudest form. The death rate of London to-day is in no sense a measure of its sanitation. This will be referred to again in a subsequent paper.



I said earlier in this paper that Farr's law did not stand alone. In later papers certain examples of similar relationships will be referred to, but one specially is mentioned here. It is given in a remarkable communication by Mr A. E. Kennealy<sup>1</sup> entitled "An Approximate Law of Fatigue in the Speeds of Racing Animals." This came into my hands a number of years ago and it immediately suggested "Farr's Law," but the difficulty which was still unsolved was as already mentioned the measure of the death rates. Mr Kennealy's paper contains the results of an investigation into the speeds of animals. It is shown that each racing record whether for horse or man, trotting, pacing, walking, running or swimming, obeys a formula of the same form. The figures compared in each instance are the record times achieved for each different distance. As is well known the rate of running for a hundred yards is greater than that for a mile, but that the record time for 20 yards, 40 yards, 100 yards, one mile, ten miles, etc., for each separate sport for practically all the racing records of the world should be comprehended in the same formula

$$T = cL^{\frac{9}{8}},$$

when  $T$  is the time taken to cover a distance  $L$ , and  $c$  is a constant, was hardly to be expected. The constancy of the value  $m = 9/8$  is surprising.

This formula when  $V$  is the average velocity can be put in the form

$$V = cL^{-\frac{1}{8}} \text{ since } V = \frac{L}{T}.$$

In this form it may perhaps represent the same kind of relationship as Farr's formula.

These remarks suggest a fact which is fully discussed in a succeeding paper that the death rates of different age periods of life in different districts are really organically connected and cannot be compared without the exercise of great care, though on superficial observation they seem directly significant. It is interesting to note that this fact was perceived by the genius of Dr Farr fifty years before modern statistical methods had been introduced.

<sup>1</sup> *Proc. Amer. Acad. Arts and Sciences*, 1906, p. 275.

TABLE I.

Showing the figures relating to Density and death rate. 1861-70\*.

No. of districts	Density (persons per square mile)	Corrected death rate (1)	Do Fitted by least squares	Crude death rate (2)	Do. Fitted by Farr	Life table death rate (3)	Do. Fitted by least squares
53	166	15.30	16.70	16.75	18.90	19.90	20.73
345	186	17.02	17.00	19.16	19.16	21.07	20.96
137	379	20.52	18.99	21.88	20.87	23.47	22.51
47	1718	24.35	24.03	24.90	25.02	26.09	26.19
9	4499	27.94	27.92	28.08	28.08	28.54	28.84
1	12,357	33.98	32.67	32.49	32.70†	32.67	31.92
1	65,823	40.55	42.39	38.62	38.74	37.17	37.74
			$E\% = 3.79$ $\Delta = 1.17$			$E\% = 2.70$ $\Delta = .90$	$E\% = 2.01$ $\Delta = .61$
(1) $R = 7.534 D^{-1.5571}$ .			(2) $R = 10.234 D^{-1.1998}$ .	(3) $R = 12.419 D^{-1.0018}$ .			

$E\%$  = mean percentage error.  $\Delta$  = square root of the mean of the squares of the errors.

\* Farr, *Vital Statistics*, p. 175.

† A misprint in the original of 37.7 has been corrected.

TABLE II.

Showing the figures relating to density and death rate. 1891-1900\*.

1	2	3	4	5	6	7	8	9
No. of districts	No. of inhabited units divided by 1,000	Density (persons per square mile)	Corrected death rate	Do. Fitted by least squares (1)	Crude death rate	Do. Fitted by least squares (2)	Life table death rate	Do. Fitted by least squares (3)
27	305	136	11.63	13.06	14.20	14.16	17.38	17.18
112	1676	161	12.54	13.43	15.05	14.51	18.01	18.12
121	2496	181	13.44	13.70	15.44	14.68	18.62	18.33
92	3849	261	14.52	14.56	15.46	15.38	19.36	19.02
53	2272	407	15.53	15.68	16.08	16.28	20.05	19.90
56	2577	457	16.53	15.99	16.67	16.52	20.24	20.13
31	1839	737	17.58	17.32	17.64	17.56	21.45	21.12
40	3690	1303	18.53	19.05	18.04	18.88	22.10	22.31
31	3159	1705	19.42	19.93	18.61	19.54	22.71	22.99
21	2240	2339	20.37	21.00	19.50	20.35	23.36	23.72
18	2777	4424	21.56	23.37	20.21	22.08	24.18	25.31
13	2119	4884	22.36	23.76	20.69	22.35	24.72	25.56
6	801	4194	23.48	23.16	22.05	21.93	25.49	25.10
5	762	2925	24.33	21.80	23.29	20.94	26.07	24.21
5	791	7480	26.54	25.51	24.74	23.60	27.58	26.68
4	288	55,563	34.82	35.66	32.67	30.49	33.25	32.58
			$E\% = 4.3$ $\Delta = 1.05$			$E\% = 3.8$ $\Delta = 1.14$	$E\% = 2.03$ $\Delta = .63$	
(1) $R = 12.40 D^{-1.6715}$ .			(2) $R = 13.57 D^{-1.2755}$ .	(3) $R = 10.83 D^{-1.0078}$ .				

\* Dr Tatham: *Decennial Supplement, Registrar-General of England and Wales*, Part II, 1908, p. lxxi.

# STUDIES IN THE MEANING AND RELATIONSHIPS OF BIRTH AND DEATH RATES.

## III.

### *The Constitution of a Death Rate.*

By JOHN BROWNLEE, M.D., D.Sc.

(With 1 Chart.)

THIS is a particularly difficult branch of the subject to investigate. It might be thought at first sight that the death rates at different age periods might be compared, and this is often done; but when it is noted that the death rates at different age periods are organically connected, it is obvious that such a comparison is statistical or actuarial and completely neglects the biology of the subject. To illustrate the problem a diagram is given. This is constructed on a principle open to objection, but, if that is remembered, it illustrates a number of points. The healthy district life table  $H_2$  has been taken as a standard of comparison. Now a healthy district life table labours under certain disadvantages. A district may, as the Register General says, be excluded because it contains an institution drawing its inmates from a wider district. This is not a serious objection. A more serious objection is the fact that whether deaths which might be considered to belong to the district are returned to it or not, the result is equally unsatisfactory. Everyone who has had practical experience knows many instances in which it is impossible to allocate a death to the district to which it properly belongs. When all is balanced, I think that probably on the whole the healthy districts get credit for more than their share of deaths, that is, for more deaths than would occur if there was no process of intermingling of town and country. For one death the town gets credit for, the country gets credit for another, in the one case some old person dying of a cancer in a hospital, in the other, some poor young person, who having tried city life, returns broken, to die of phthisis or some similar disease. Thus a healthy district life table is open to objection as a criterion. It is, however, for the purpose at present required, the only one at our disposal.





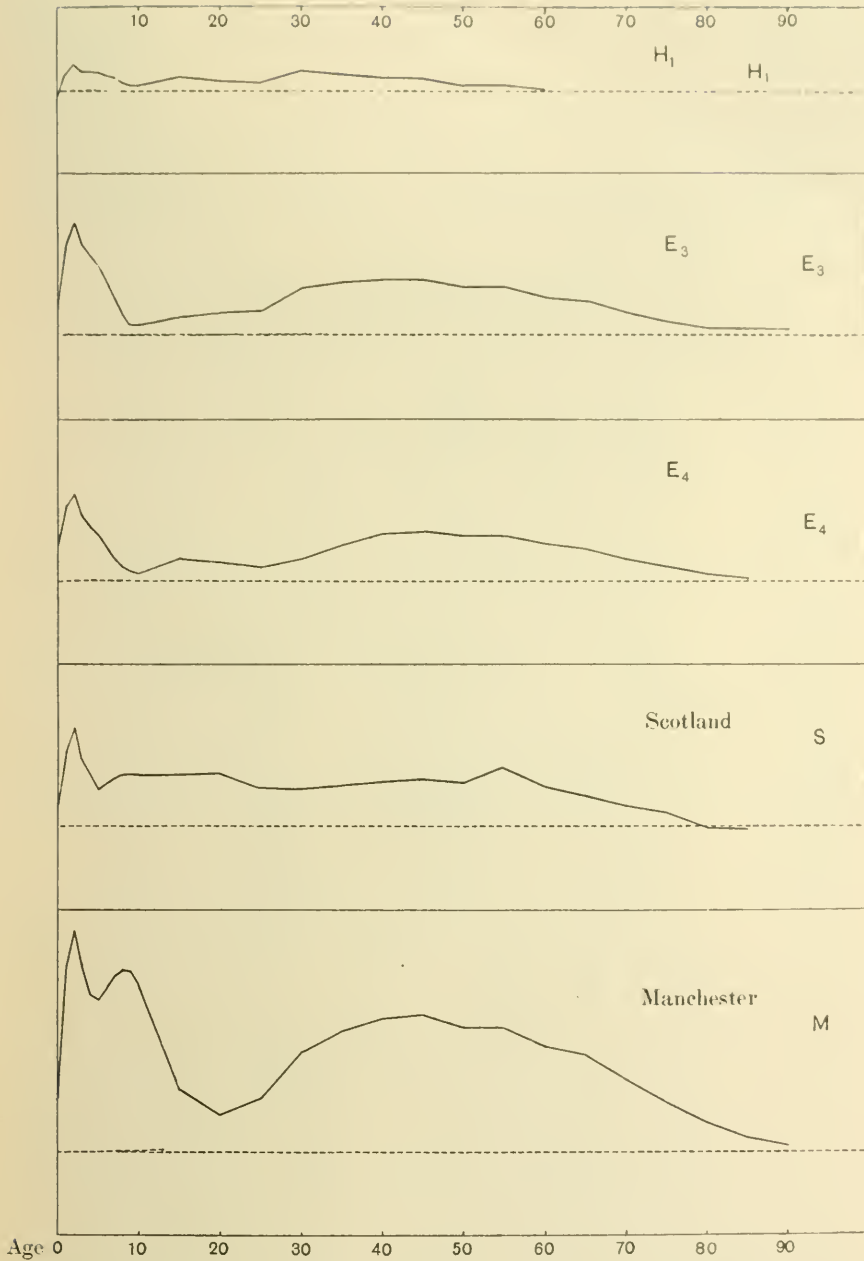


Diagram showing graphically the relationships of some of the figures given in Table I.

The method employed is as follows: A series of life tables have been taken. The death rates have been calculated for each year of age of life from 0 to 10 and thereafter at quinquennial intervals 15, 20, 25, etc. The death rates of the life table  $H_2$  at each age have been taken as unity and all the other tables reduced in this proportion. These comparative rates are given in Table I for the life tables  $H_1$ ,  $E_3$ ,  $E_4$ , Scotland, Salford and Manchester, and may be there studied at leisure. For ease of observation the values for the male sex alone have been graphically expressed in the Diagram. It will be noticed that the general form of the curves is the same. There are two maxima in each case, one between the ages of two and three years, and another about the age of forty. This is observed in every case; it is not an arbitrary phenomenon due to district. It is true that each life table has points of difference more or less special to itself, but the main features are the same. Now this clearly means that life as a whole responds in quite a definite manner to healthy or unhealthy surroundings. An excess of mortality in the first year of life is followed by a greater excess in the third, and an excess of mortality in the twentieth year is followed by a correspondingly greater excess in the fortieth. What appears a trivial increase of the death rate at the age of twenty is, however, a certain prognostic of something far from trivial when the age of forty is reached. For this low mortality in towns at twenty years of age is not due to immigration from the country. Such immigration is much less than that required to produce the defect. It is an expression of a phase in the relations of life to its surroundings, a phase not to be isolated and considered separately.

How then is it possible to form any estimate as to the extent a death rate differs from what might be expected from the general health or want of health of the district? I think it can be done to a certain extent by a process of prediction.

The tables  $H_2$ ,  $H_1$ ,  $E_4$ ,  $E_3$  have all been constructed by the same process, and they all are based on large populations, the two latter on the whole population of England. They represent the most definite information that can be obtained from the country at present. Taking the reciprocal of the expectation at birth, or the life table death rate ( $R$ ) as the standard of comparison, the unit to which all is to be reduced, and denoting the death rate at any age  $x$  as  $r_x$ , a relationship  $r_x = mR + c$  (when  $m$  and  $c$  are constants) is assumed. This assumption is justified by the results; it is only one of many other relationships of the same

kind which are found to be described almost truly by straight lines. The values obtained are given in Table II.

These values of  $m$  and  $c$  are used to predict the corresponding mortalities at all ages in four different countries and towns: Scotland, Manchester, London and Salford. In predictions like these a 10 per cent. variation might easily be observed, accounted for, firstly, by the roughness of the method, and secondly, by the processes employed in smoothing crude statistics to form life tables. In all instances

TABLE II. *Showing the values of constants  $m$  and  $c$  used for predicting death rates at different ages.*

Age	Males		Females	
	$m$	$c$	$m$	$c$
0	14.95	- 155.33	15.46	- 179.24
2	3.07	- 47.25	3.45	- 51.10
4	1.18	- 16.10	1.43	- 19.83
6	.459	- 4.81	.517	- 5.59
8	.123	.25	.063	1.61
10	.009	1.85	-.091	3.89
15	.114	.39	-.018	3.45
20	.204	.07	.100	2.46
25	.255	.27	.236	.63
30	.484	- 3.44	.473	- 2.78
35	.796	- 8.47	.727	- 6.78
40	1.17	- 14.35	.991	- 10.61
45	1.52	- 19.16	1.26	- 14.50
50	1.89	- 24.00	1.55	- 17.38
55	2.50	- 30.83	2.00	- 21.58
60	3.14	- 34.98	2.55	- 24.35
65	3.97	- 38.98	3.27	- 26.39
70	4.50	- 27.91	4.00	- 20.24
75	4.56	6.75	4.27	7.04
80	3.85	77.39	4.00	61.57
85	2.00	199.22	2.91	154.30

the average percentage error is much below this. The correspondence is in fact so close that it must be assumed that the variations of the death rates at different ages are organically connected. As regards the results it is found that for practically any age, at ages above twenty-five years, the theoretical death rate predicted by this method corresponds with the actual in the range of experimental error. Under twenty-five years certain differences make themselves apparent. If the infantile death rate predicted is found to be equal to that actually observed, the correspondence between the predicted and actual values of the death rate for the whole life is very close. Examples of this

are shown in the life tables for London and Salford for the decade 1891-1900. In London the correspondence is almost absolute, except at the age of 10. In Salford it is not nearly so absolute, but as the actual figures for Salford are not so continuous, due apparently to the small numbers on which the table is based, individual differences are larger. The special discrepancy about the age of 10 in both instances is probably due to the fact that in the neighbourhood of this age the minimum death rate exists, and in the neighbourhood of a minimum the methods of life table approximation are open to special error.

Taking the life table for Scotland, a different condition of affairs is seen. Scotland varies markedly from England in the comparative absence of summer diarrhoea, with the result that the infantile death rate predicted from the English life tables is twenty per thousand in excess of the actual infantile death rate of Scotland. For the next few years till the age of six is attained there is no appreciable difference between the actual and the predicted death rates, but this defect in the infantile death rate is balanced by the excess of the actual death rate over the theoretical between that age and the age of thirty, after which the population in Scotland shows essentially the same mortalities as the English tables, used in the manner described, predict.

Of the same phenomenon, Manchester affords a striking example, in curious distinction to the neighbouring town of Salford. In this case the predicted infantile death rate is 55 per thousand in excess of that observed. The period of life at which the compensation begins is from one to two years earlier than that in Scotland, the actual death rate at six years of age being twenty-five per cent. in excess. Matters adjust themselves also at a somewhat earlier age, since by the time twenty years is attained, the actual and theoretical death rates have come into correspondence. More especially when the township of Manchester and the outlying townships are severally examined, the same phenomena are observed, the points of difference not being sufficiently important to require special comment.

It is to be noted that the greater mortalities in Scotland and Manchester at the ages, approximately, of from six years to twenty-five years, though apparently excessive as rates in the sum correspond almost exactly to the number of deaths represented by the deficiency of the actual from the theoretical mortality at the age 0-1.

With regard to the mortalities of the adult population, it is evident from what has been said that practically the same result can be obtained

TABLE III. *Showing actual and predicted death rates, male and female, for London, Salford, Scotland and Manchester.*

	London				Salford			
	Actual		Theoretical		Actual		Theoretical	
	Male	Female	Male	Female	Male	Female	Male	Female
0	207.99	170.31	209.56	161.81	302.50	244.97	297.79	240.81
2	28.01	27.40	27.65	25.01	40.40	38.01	45.76	42.64
4	12.38	12.62	12.57	11.72	15.21	18.16	19.50	19.02
6	6.02	6.19	6.39	5.82	6.71	7.02	9.10	8.46
8	3.15	3.38	3.26	3.00	4.60	5.00	3.98	3.32
10	2.26	2.44	2.06	1.88	3.19	3.52	2.12	1.42
15	3.12	2.79	3.16	3.05	3.60	3.61	3.83	2.96
20	4.13	3.19	5.04	4.67	6.61	4.50	6.24	5.18
25	5.24	4.03	6.48	5.84	7.52	5.63	7.99	7.01
30	7.54	5.82	8.37	7.65	8.15	7.93	11.23	10.07
35	11.26	8.62	10.96	9.25	9.95	10.95	15.66	12.97
40	14.73	11.07	14.25	11.25	18.96	14.58	21.17	16.32
45	18.40	13.52	17.83	13.30	24.92	18.87	26.77	19.73
50	23.91	17.65	22.16	16.81	30.43	24.48	33.32	24.73
55	31.57	23.55	30.25	22.54	40.10	33.97	45.02	32.76
60	42.49	32.14	41.64	31.90	62.10	49.62	60.16	44.93
65	58.16	45.39	57.85	45.75	79.14	66.16	81.26	62.46
70	83.21	67.28	81.97	68.00	100.68	94.28	108.54	88.44
75	120.67	101.16	118.03	101.24	189.38	168.50	144.94	123.06
80	173.26	150.29	171.26	149.81	161.66	129.02	193.96	170.25
85	242.87	218.02	248.02	218.49	228.18	314.40	259.82	233.36

	Scotland				Manchester			
	Actual		Theoretical		Actual		Theoretical	
	Male	Female	Male	Female	Male	Female	Male	Female
0	158.97	127.89	179.05	146.35	219.44	174.97	275.51	223.03
2	22.38	22.13	21.39	21.56	37.53	36.25	41.19	38.67
4	9.39	9.75	10.17	10.29	17.09	17.44	17.75	17.38
6	5.57	5.89	5.45	5.30	11.08	11.63	8.41	7.86
8	4.03	4.47	3.00	2.94	8.07	8.30	3.80	3.25
10	3.18	3.62	2.05	1.97	6.05	6.06	2.10	1.52
15	3.86	4.66	2.93	3.07	4.26	3.97	3.66	2.98
20	6.27	5.68	4.63	4.57	5.54	5.06	5.94	5.06
25	7.27	6.57	5.96	5.60	8.36	7.47	7.61	6.77
30	7.65	8.18	7.38	7.18	11.79	10.20	10.50	9.53
35	9.38	9.45	9.34	8.53	15.64	12.76	14.47	12.14
40	11.68	10.52	11.86	10.26	20.08	15.45	19.42	15.18
45	14.72	11.93	14.73	12.04	25.22	19.22	24.51	18.29
50	19.12	15.60	18.30	15.26	31.79	25.22	30.50	22.95
55	28.33	21.23	25.15	20.54	41.80	34.12	41.29	30.46
60	36.87	29.53	35.23	28.35	57.39	47.27	55.49	42.00
65	50.40	44.20	49.75	42.48	80.12	68.58	75.34	58.70
70	71.82	61.03	72.78	64.00	111.01	94.98	101.83	83.84
75	111.59	89.04	108.73	96.97	152.36	132.37	138.14	118.15
80	145.13	133.73	163.42	145.81	206.31	178.34	188.23	165.65
85	222.09	204.16	243.94	215.58	275.28	233.10	256.84	230.02



in several different ways. On general principles, a high infantile mortality—granted similar environmental conditions—will cut off a good many children, who would otherwise perish at early ages, but the fact which is most evident from the tables given here, is that mortality is specially distributed and that life at all ages is acted on by unhealthy surroundings in a way which is very closely correlated with the sum total of the depressing influences due to the environment. The conditions which produce a high infantile death rate are exactly those conditions which depress the vitality of the whole adult population. All the evidence is against the view that a high infantile mortality produces a healthier population at adult ages. The fact that in the cases of such towns as London and Salford the death rates at all ages can practically be predicted from the knowledge of infantile death rate alone, shows the danger of such a method of reasoning. It is quite true that in certain places, such as Manchester and Scotland, the saving of infantile life is associated with higher death rates in the immediately succeeding ages. But in adult life the influence on health in the case of Manchester is just as adverse as in the case of Salford.

## STUDIES IN THE MEANING AND RELATIONSHIPS OF BIRTH AND DEATH RATES.

### IV.

*On the Range of Instances in which Geometrical Progressions describe numerically processes of life, i.e. those processes which might be explained by a monomolecular reaction.*

By JOHN BROWNLEE, M.D., D.Sc.

In recent years it has been found that *in vitro* many ferments, etc., lose their power of action at a rate corresponding to the monomolecular reaction, i.e. if the amount of the substance present at the beginning is denoted by unity, and, if after a certain period of time only one half of the substance remains active, then it may be predicted that at the end of an equal period of time, one half of one half, or only one quarter of the original amount, will still retain its power of action. It is not necessary to give examples, they are sufficiently well known.

In actual living organism, however, this relationship can be just as well demonstrated in a large number of instances, some immediately obvious, others requiring some care to establish. The oldest known example, discovered by Gompertz, is the increase of the death rate with age. Thus the average death rate among males (1858-1901) as given by the Registrar-General is for the age period 55-65, 33.3 per mille, for 65-75, 68.3 per mille, 75-85, 147.4 per mille, and for the ages above 85 as 308.6 per mille.

It will be noticed that the death rate approximately doubles itself with each ten years' increase of age. For high ages in fact this relation is an excellent interpolation formula.

It is not necessary to give in this place cumulative evidence as all that is required for the present purpose is to show that the phenomenon is of some generality. As typical examples it is to be noted

that the following sequence of values are described by a geometrical progression:

(1) The rate of decrease in the case mortality of infectious diseases among children as age increases.

(2) The rate of increase in the case mortality of infectious diseases among adults as age increases.

(3) The rate of decay of the protective power of vaccination as age increases.

(4) The decay of the natural immunity of children and the growth of natural immunity of adults towards certain diseases as age increases.

(5) The loss of infectivity of an organism during an epidemic.

Some of the data referring to these instances ("The Relation of the Monomolecular Reaction to Life Processes and to Immunity," *Proc. Roy. Soc. Edin.* 1911) have already been published, but the figures referring to scarlet fever are reproduced.

The case mortality from scarlet fever is highest in children between one and two years, and thereafter declines from year to year. The rate of decline is such that the case mortality during each year of life is three-quarters that of the preceding, as can be easily seen from the table (Table I) in which the case mortalities taken from the statistics of Glasgow and Manchester are given in parallel columns with the appropriate theoretical values, these being the only two cities for which statistics can be given for the several years up to ten. Though the type of scarlet fever prevalent among children in Glasgow is considerably more severe than that in Manchester, it is worthy of special notice that the ratio of decrease is identical. The case mortality for measles varies in the same way, but in this instance the ratio of diminution between the case mortalities of succeeding years of life is not  $\cdot75$  but  $\cdot65$ ; that is, children tend to grow out of the fatal period more quickly. Thus after three years the susceptibility to death in the case of scarlet fever has only fallen to  $\cdot42$  of that of the epoch of commencement, while in the case of measles it has fallen to  $\cdot28$ .

To these examples the figures relating to diphtheria are added, as they have not been shown to obey the same law. The case mortalities are those obtaining in the city of Manchester for the ten years 1893-1903. In Table I these figures and the progression fitted by the geometrical law are given in parallel columns. The ratio found in this case between the mortality of successive years of age is conspicuously higher, namely,  $\cdot86$ , a figure much higher than in the cases of measles and scarlet fever.



TABLE I.

*Showing the case mortalities of scarlet fever and diphtheria fitted to curves of the form  $y = ae^{-kx}$ .*

Age period	Scarlet Fever		Diphtheria	
	Glasgow	Manchester	Manchester	
	Actual	Theoretical	Actual	Theoretical
1- 2	24.3	22.2	19.3	19.3
2- 3	16.5	16.5	14.7	14.2
3- 4	12.6	12.4	12.2	10.6
4- 5	9.1	9.3	8.7	7.8
5- 6	7.0	7.0	5.7	5.8
6- 7	4.1	5.2	4.5	4.3
7- 8	3.6	3.9	3.4	3.2
8- 9	3.1	2.9	2.2	2.3
9-10	2.2	2.2	—	—
10-15	—	—	—	—
15-20	—	—	—	—

II. In this group of instances the death rate steadily increases with age. The figures for smallpox and typhus have already been published and the only point of importance is the ratio of increase of the case mortality with age. With smallpox in Gloucester, the case mortality increases with each ten years of age in the ratio 1:30. With regard to typhus the ratio is the same for Glasgow and London and is considerably larger than that just given, namely 1:46.

In more extreme age diarrhoea furnishes a good example.

For the age periods 45-55, 55-65, 65-75, 75- , the death rates per million living during the decade 1891-1900 were respectively 95, 243, 715, 2151 for males, and 76, 226, 703, 2011 for females, showing a rough ratio between successive numbers of nearly 3, the greatest ratio hitherto found. Diarrhoea is an infectious disease which is ubiquitous, and it may be taken that all are alike exposed to infection. This curve thus represents the combined effects of susceptibility to disease and case mortality.

Of a like nature is the phenomenon shown by the influenza statistics. As this disease caused no mortality at all in London in the late eighties, the figures relating to the deaths may be taken as practically true when the years of the great epidemics 1890, 1891 and 1892 are considered. It may be considered that all ages alike succumbed to infection and that the death rates at different ages are thus equivalent to case mortalities. When the ratio of increase is examined, however, it is found to be practically identical in its value with that occurring between the death rates from all causes, given earlier in the paper. With each

ten years of age from 45 upwards, the death rate, both for males and females, practically doubles itself. Influenza may thus be looked upon more as a factor accelerating death than as a disease with a special mortality of its own. The figures are given in the adjoining table.

TABLE III.

*Death rates per thousand at different ages from influenza for London during the years 1890, 1891 and 1892.*

Age period	Death rate per thousand	
	Male	Female
45-55	·79	·59
55-65	1·33	1·28
65-75	2·64	2·59
75-	5·61	5·07

III and IV. The figures regarding the decay of the protective power of vaccination treated by the method of correlation have already been published<sup>1</sup>, and in this place the ratio of decay is measured in a different manner. An analysis of the susceptibility to certain diseases at each age is made. It involves a double problem; firstly, in youth, either owing to special insusceptibility to disease, *e.g.* enteric fever, or to acquired insusceptibility, *e.g.* smallpox after vaccination, there is a period in which the cases of the disease are few; secondly, as age advances a special immunity develops. It is apparently a fact, and a fact somewhat curious, that these two forms of immunity are additive, or that the protection at any moment is proportional to their sum. Three examples are considered. In the first place the age distribution of susceptibility to smallpox among the vaccinated is examined. The most important data for this are contained in Dr Barrie's report on the epidemic of smallpox in Sheffield in the year 1887. Here owing to the fact that a census was taken as to the numbers of vaccinated in the whole population, the susceptibility to smallpox of persons at different ages can be calculated. This is exceedingly important because owing to the manner in which towns recruit their populations from different districts, very marked variations in the number of vaccinated and unvaccinated at different ages may occur. The drawback to Dr Barrie's Census is that he has not separated between those who are definitely and those who are doubtfully vaccinated as is usually done. There are thus far too many children under five years of age as compared with the statistics of other places. Some correction has therefore to be made to obtain the probable number of those who

<sup>1</sup> *Loc. cit.*

were definitely vaccinated. This is done on the basis of the Glasgow epidemic by assuming that from 0 to 5 years 45 per cent. of the total cases occurred among definitely vaccinated children and from 5 to 10 years 90 per cent. At the other ages correction is immaterial as insusceptibility to smallpox depends much more upon the natural immunity due to age than on the protection due to vaccination.

Two factors determine the distribution of susceptibility, one, the protection due to vaccination great in youth and gradually disappearing, the rate of its disappearance being described by the terms of a geometrical progression: the other factor, the increase of natural immunity with age also described in a like manner. The fitting of this compound curve to the statistics is largely a matter of trial and error. In the accompanying table (Table V) are shown in parallel columns the total number of cases; the susceptibility at each age period; then the reciprocal of the latter: in the next two columns this analysed into its two parts: these two latter columns summed: the number of cases to which they correspond calculated and compared with the number given by observation. The difference is remarkably slight; when the usual calculations are made it is found that  $\chi^2 = 3.24$ , which gives the probability of the analysis  $P = .95$ .

The epidemic of miliary fever in Oise in 1827 is next analysed. For this a table constructed in the manner described above is also given. It is found that  $\chi^2 = 6.0$ , which gives a probability  $P = .53$ . Though not so high as that shown for smallpox, it must be accounted a good fit.

The cases of enteric fever in London are given as the last example and though they do not give a good fit ( $\chi^2 = 18.16$ , due to the very large number of cases analysed, over five thousand) yet the curve is essentially similar and the divergence much less than that found by Prof. Pearson in his analytical graduation of the curve. We thus have the susceptibility to certain diseases described by a curve of different form to any hitherto proposed. The graduation which has been discussed is obviously described by the formula

$$y = \frac{a}{e^{-mx} + e^{nx}}.$$

It is thus possible to describe these three diseases in a manner capable of being explained by the monomolecular reaction. The manner in which the ratios of decay and growth of immunity vary is exceedingly interesting and the collected results are given in the accompanying table.

TABLE IV.

	Rate of decay of the immunity of youth per 5 years	Rate of growth of old age immunity per 10 years
Smallpox .. .. .	.192	1.91
Miliary fever .. .. .	.523	1.35
Enteric fever .. .. .	.182	2.15

It is to be noticed that each disease has its special method of variation.

It may be remarked that though these adult diseases have been thus easily analysed others have more complex immunity phenomena resembling in this respect the diseases of childhood.

These will be considered later after the method of treating a sum of monomolecular reactions has been described.

V. It is not necessary to discuss the curve of an epidemic in this place but only to notice that it can be approximately accounted for if the organism loses its infectivity at a rate something approaching a geometrical progression.

## APPENDIX.

$$\text{On the curve } y = \frac{a}{be^{-mx} + ce^{nx}}.$$

With change of origin this equation can be put in the form

$$y = \frac{ae^{px}}{e^{-mx} + e^{mx}}.$$

The curve thus contains three constants and is of some interest. It allows finite susceptibility at any age and describes the course of immunity during life in a definite formula. It is therefore superior to graduation curves which seek to smooth merely the numbers of cases and tell nothing of what is going on. To calculate the area and moments the term in the numerator is expanded and each term severally integrated from positive to negative infinity. Thus

$$\begin{aligned} \int_{-\infty}^{\infty} \frac{e^{px} dx}{e^{-mx} + e^{mx}} &= \int_{-\infty}^{\infty} \frac{dx}{e^{mx} + e^{-mx}} + p \int_{-\infty}^{\infty} \frac{x dx}{e^{mx} + e^{-mx}} \\ &\quad + \frac{p^2}{1.2} \int_{-\infty}^{\infty} \frac{x^2 dx}{e^{mx} + e^{-mx}} + \dots \\ &= 2 \left\{ \frac{\pi}{2m} + \frac{p^2}{1.2} \left( \frac{\pi}{2m} \right)^3 E_1 + \frac{p^4}{1.2.3.4} \left( \frac{\pi}{2m} \right)^5 E_2 + \dots \right\} \\ &\quad \text{where } E_1, E_2, \text{ etc., are Euler's numbers,} \\ &= \frac{\pi}{m} \sec \frac{p\pi}{2m}. \end{aligned}$$



TABLE V.

Showing the fitting of curves of susceptibility to the form  $\eta = \frac{a}{be^{-mx} + ce^{nx}}$ .

(a) *Smallpox, Sheffield, 1887-8*:

Age period	No. of cases	Susceptibility per 1000	Reciprocal	Analysis	Sum	Theoretical no. of cases
0-5	431	253	88.844	.752	91.973	41.1
5-10	215.5 <sup>1</sup>	603	18.518	1.038	18.584	214.8
10-15	782	2067	4.837	1.433	3.375	787.0
15-20	1240	3987	2.508	1.978	.649	1184.0
20-25	950	3480	2.873	2.731	.125	956.0
25-35	1019	2194	4.558	4.438	.024	1041.0
35-45	429	1182	8.460	8.460	.005	428.0
45-55	156	620	16.129	16.129		156.0
55-65	66	3033	33.045	30.740		68.4

<sup>1</sup> Corrected as described in the text.

$$\chi^2 = 3.24.$$

$$P = .91.$$

(b) *Miliary fever, Oise, 1827*:

Age period	No. of cases	Susceptibility per 1000	Reciprocal	Analysis	Sum	Theoretical no. of cases
0-10	77	11.3	88.496	2.385	80.000	82.7
10-20	205	39.3	25.445	3.233	21.909	207.9
20-30	429	101.9	9.814	4.381	6.000	405.5
30-40	501	128.3	7.811	5.938	1.642	516.6
40-50	410	119.9	8.340	8.047	.449	402.5
50-60	208	98.8	10.111	10.905	.123	190.7
60-70	101	73.7	13.569	14.778	.033	92.5
70-80	15	33.0	27.076	20.027	.010	20.2

$$\chi^2 = 6.21.$$

$$P = .51.$$

(c) *Enteric fever, London, 1871-93*:

Age period	No. of cases	Susceptibility per 1000	Reciprocal	Analysis	Sum	Theoretical no. of cases	Prof Pearson's distribution
0-5	266	530.3	18.858	.636	18.150	267	353
5-10	1143	2516.7	3.974	.932	3.300	1072	1236
10-15	2019	4848.4	2.063	1.386	.600	2097	1833
15-20	1955	4690.3	2.131	2.031	.090	1964	1735
20-25	1319	3078.5	3.248	2.975	.019	1426	1374
25-35	1360	1895.4	5.275	5.275	.003	1360	1433
35-45	462	889.1	11.248	11.367	.000	457	470
45-55	138	374.4	27.710	24.405	.000	156	136
55-65	22	121.9	82.056	51.400	.000	42	201

Present fitting:  $\chi^2 = 22.3$ ,  $P = .005$ ; Prof. Pearson's fitting:  $\chi^2 = 83.1$ ,  $P = 0$ .

<sup>1</sup> For 55 to 60 as against 14.

The moments about the origin are obviously obtained by differentiating this with regard to  $p$ . Transferred to the centroid vertical, which is at a distance

$$\frac{\pi}{2m} \sec \frac{p\pi}{2m} \tan \frac{p\pi}{2m}$$

from the origin, the values are

$$\mu_2 = \left(\frac{\pi}{2m}\right)^2 \sec^2 \frac{p\pi}{2m},$$

$$\mu_3 = 2 \left(\frac{\pi}{2m}\right)^3 \sec \frac{p\pi}{2m} \tan \frac{p\pi}{2m},$$

$$\mu_4 = \left(\frac{\pi}{2m}\right)^4 \left(4 \sec^2 \frac{p\pi}{2m} \tan \frac{p\pi}{2m} + 5 \sec^4 \frac{p\pi}{2m}\right).$$

Whence

$$\beta_1 = 4 \sin^2 \frac{p\pi}{2m},$$

$$\beta_2 = 4 \sin^2 \frac{p\pi}{2m} + 5,$$

$$\begin{aligned} F &= 2\beta_2 - 3\beta_1 - 6 \\ &= 4 - 4 \sin^2 \frac{p\pi}{2m}. \end{aligned}$$

The curve would thus be very easily fitted by the moments were it possible to obtain these from the statistics. It has the moment relationship of Type IV or Type VI according as  $p < \text{or} > \frac{m}{3}$  approximately. The curve in general will be asymmetrical because the ratio of the decay of the natural immunity of childhood is not identical with that at which immunity increases with age.

# STUDIES IN THE MEANING AND RELATIONSHIPS OF BIRTH AND DEATH RATES.

## V.

*On the difficulty that in applying the laws of physical chemistry to life processes, indices occur which suggest the actions of fractions of a molecule.*

By JOHN BROWNLEE, M.D., D.Sc.

IN a large number of cases the curves which describe the rate at which ferments, antibodies, etc., disappear in the body accord in form with the equations of physical chemistry, but not so as to be readily interpretable. If one molecule, two molecules, etc., take part in a reaction then it is clear that the indices in the equations must bear certain relations to whole numbers. Failure to conform to these relations seems at first sight to imply that a fraction of a molecule takes part in a reaction, but this is not necessarily the case. Such a failure has been found to appear when the curves of many vital phenomena, *e.g.* the disappearance of agglutins in an organism, are fitted to physico-chemical equations. A possible meaning, however, can easily be seen.

Firstly, as the monomolecular reaction is so frequent in life phenomena, it is quite reasonable to assume that it is the rule. Secondly, it is highly improbable that such a substance as an agglutin is a simple substance. It is much more probably a group of substances each of which has its own rate of decay. If each member of this group be taken to obey the monomolecular reaction, the amount of agglutin present at a definite time  $x$ , instead of being represented by the form  $ae^{-kx}$  as is required by the monomolecular theory, will be represented by a sum of such forms, each substance disappearing at a different rate, and each present originally in a different amount.

Now certain curves of frequency exist which may be tried. If the frequency of each value of  $k$  be denoted  $a\kappa^n e^{-\gamma\kappa}$  (Pearson's Type III, a curve having a very wide range of forms) the amount of the substance after a period  $x$  will be represented by

$$a \int_{\kappa}^{\infty} e^{-\kappa x} \kappa^n e^{-\gamma\kappa} d\kappa,$$



or ( $M$  being a constant)

$$\frac{M}{(x + \gamma)^{n+1}} \dots \dots \dots (A),$$

which is the form required by a multimolecular reaction where  $k+1$  is equal to  $\frac{1}{p-1}$ ,  $p$  being an integer. But on the theory here developed the values of  $n$  are not circumscribed in this way, and  $p$  may be any number, fractional, or integral. Thus a possible explanation of a common phenomenon is obtained.

It is to be noted that the rates of the disappearance of the agglutins or the precipitins formed when the same organism is inoculated into different animals vary greatly, implying that the amount of each separate agglutinin with its special rate of decay may be very different even in nearly allied forms. The experimental working of this subject opens a wide field.

It is not necessary to quote examples that are familiar to all workers in this subject. To illustrate the range, however, an example from the statistics of children's diseases, namely the death rate from whooping cough, has been chosen, as death from whooping cough is due to a considerable variety of causes—convulsions, broncho-pneumonia, etc.

In the accompanying table the numbers of admissions and of deaths, and the case mortality of persons suffering from whooping cough, treated in the City of Glasgow Hospital, Belvidere, between the years 1885 and 1902 are given. The theoretical mortality is calculated on the hypothesis above stated. The theoretical numbers of deaths, calculated from the number of cases, which being the larger number

*Table showing that the case mortality of cases of whooping cough from one year to ten is completely described by the curve  $y = \frac{4090}{(4.7 + x)^{2.638}}$ , where  $y$  is the case mortality and  $x$  the age in years.*

Age	No. of cases	No. of deaths		Case mortality	
		Actual	Theoretical	Actual	Theoretical
1- 2	619	233	233	38.0	38.0
2- 3	742	190	183	25.6	24.6
3- 4	779	119	130	15.3	16.7
4- 5	695	81	85	11.7	12.2
5- 6	585	53	53	9.1	9.1
6- 7	420	28	29	6.6	7.0
7- 8	228	13	13	5.7	5.5
8- 9	112	6	5	5.3	4.4
9-10	55	2	2	3.6	3.6

has the smaller probable error, are added to admit of statistical comparison. The correspondence is exceedingly close since  $\chi^2 = 1.9$  and  $P = .99$ . This is not a solitary example, and others will be discussed later when some of the problems of special diseases are considered.

In what has gone before, the distribution of the value of  $k$  has been assumed to be that of Type III. It may, however, be at least equally probably assumed to be normal. In this case, the resulting equation giving the amount of the original substances present at the time,  $x$  is of the form:

$$y = a \int_{-\infty}^{\infty} e^{-\kappa x} e^{-\frac{(\kappa-k)^2}{2\sigma^2}} d\kappa,$$

$k$  being the mean value of  $\kappa$ , or when integration is effected:

$$y = Me^{-kx - \frac{1}{2}\sigma^2 x^2},$$

$M$  being a constant. If the range of  $\kappa$  is large, *i.e.* if  $\frac{k}{\sigma}$  is small, the deviation from the simple exponential curve is considerable. If, however, the range of  $\kappa$  is small, *i.e.* if  $\sigma \approx 0$ , the resulting formula is approximately the simple exponential. A certain variation in the value of  $\kappa$  may, therefore, take place, without the result of the experiments being found to diverge much from the monomolecular law.

A simple case which may be of interest is that of a mixture of two substances, both decaying according to the monomolecular law, of which the values of  $\kappa$  are somewhat different. To illustrate the point, imagine the sum of two geometrical progressions, one which has a ratio of  $\frac{3}{4}$  and the other of  $\frac{1}{2}$ . The figures are given below:

1.0000	.7500	.5625	.4218	.3146	.2373	.1780	.1335
1.0000	.5000	.2500	.1250	.0625	.0313	.0156	.0078
2.0000	1.2500	.8125	.5469	.3789	.2686	.1936	.1413
2.0000	1.2514	.8138	.5470	.3779	.2677	.1936	.1424

Beneath the sums of the two progressions, a series of theoretical figures are added, which have been obtained by fitting the descending series to an equation of the form:

$$y = \frac{4225600}{(10.659 + x)^{5.2285}}.$$

The correspondence is as absolute as could be expected in any series of observations made on experiments. It is obvious here that the power to which the denominator is raised is the equivalent of a reaction in which the fractions of a molecule take part.

## COMPLEMENT FIXATION IN PULMONARY TUBERCULOSIS.

By J. A. D. RADCLIFFE, M.B., B.Ch.

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THE application of the method of fixation of complement as a means of investigating immunity in tuberculosis dates from the experiment of Bordet and Gengou, in which they were able to demonstrate complement-fixing antibodies, against both avian and human tubercle bacilli, in the serum of guinea-pigs experimentally infected with avian bacilli. Gengou extended these experiments later, and showed that guinea-pigs infected with acid-fast bacilli, either pathogenic or saprophytic, developed complement-fixing antibodies not only against the homologous bacilli, but also against the other acid-fast bacteria. These observations of Gengou have been confirmed by other workers and consequently this reaction possesses only a relative specificity for tuberculosis, and must only be regarded as a group-reaction common to the acid-fast bacilli.

Wassermann and Bruck applied the method to the diagnosis of tuberculosis in man, using as antigen either old Tuberculin or the Tuberculin R. of Koch. They were, however, only able to demonstrate complement-fixing antibodies in the serum of patients who had been treated for a long time with tuberculin. Citron was the first to find these bodies in the serum of tuberculous persons who had not received any tuberculin. These results were confirmed by Lüdke, but were criticised by Morgenroth and Rabinowitsch who attributed them to "summation," and found that the serum of healthy persons might give similar results.

Numerous investigations have, since then, been carried out, both in cases of natural infection and in experimental tuberculosis, but the results have been very divergent, particularly among the earlier workers on the subject. The observers who have obtained the best

results have only succeeded in getting a positive finding in about 50 % of the cases, and generally only when the disease was in an advanced stage.

The results in bovine tuberculosis are no better, with the exception of those obtained by Hammer, who obtained 100 % of positive results in the case of 48 tuberculous bovine sera, but among 48 normal animals two were positive. Hammer used as his antigen a mixture of bovine tuberculin and an acetone-alcohol extract of tuberculous tissue. His results have not been confirmed by other workers using the same technique, *e.g.*, Bang and Andersen.

Among the more recent publications on the subject, those of Besredka and of Calmette are the most important and appear to be much more reliable than the earlier works. Besredka and his co-workers use a special egg-bouillon for the production of the antigen, and obtain about 90 % of positive results in tuberculous cases. The value of their method is, however, impaired by the fact that syphilitic sera also give positive findings, so that the method cannot be regarded as a specific one. Renaux claims to have overcome this difficulty by extracting the Besredka antigen with sulphuric ether and so removing from it the fatty bodies. This treatment is said not to affect the value of the antigen in tuberculosis, whilst it no longer gives inhibition of haemolysis with syphilitic serum.

Calmette and Massol prepare their antigen by extracting washed tubercle bacilli with a 1 % solution of Witte's peptone at a temperature of 65°, filtering and concentrating the filtrate. Using this antigen these authors have obtained 92.5 % of positive results in 134 cases of human tuberculosis. I have not, however, seen any confirmation of their results by other workers, and a few experiments carried out here did not give very reliable findings, owing to the amount of "Eigenhemmung" in the antigen.

The great disparity in the results obtained by different observers is in all probability due mainly to differences in technique, and particularly to the use of one or other of the tuberculins as antigen. It is now recognised, however, that tuberculin is not a suitable antigen for this work, and that, in addition, it possesses such marked "Eigenhemmung" qualities that errors due to summation effects are very likely to be met with.

The use of alcoholic extracts either of tuberculous tissue or of tubercle bacilli is also unsatisfactory, as these extracts may act as syphilitic antigens and so give the Wassermann reaction.



On account of these differences and uncertainties in results, it is impossible to compare the work of different observers, and consequently it is unnecessary to discuss the literature of the subject.

In this communication I have embodied (Part I) the results obtained when using an antigen which, whilst relatively easy to prepare, is less likely to give rise to errors due to unspecific absorption. In Parts II and III the effects of certain alterations in the technique are discussed.

## PART I.

### *Technique.*

The general arrangement of the test conforms to that of the original Wassermann reaction, and so does not require detailed description. It may be mentioned, however, that each substance used in the test (viz. serum, antigen, complement, haemolytic amboceptor and blood) was contained in a volume of 0.5 c.cm., so that the total volume in each tube at the end of the experiment was 2.5 c.cm.

Of the substances employed in the test, two, viz. antigen and complement, demand careful consideration, and will be dealt with in detail, the others are not subject to much variation and only require a few words of explanation.

(1) *Serum.* The serum for examination has always been obtained by pricking the thumb according to the well-known technique of A. E. Wright. By this method, which has been minutely described by Fildes and McIntosh, it is possible to obtain nearly 2 c.cm. of blood. The serum is separated from the clot by the centrifuge, and is inactivated by heating at 56° for 30 minutes. This inactivation has always been carried out within 24 hours after the withdrawal of the blood. Fresh, *i.e.* active, serum has never been employed. The maximum quantity of serum used has been 0.1 c.cm.

(2) *Haemolytic amboceptor.* This was at first a sheep-rabbit amboceptor, but in the latter half of the work, an ox-rabbit haemolysin was used. The latter is preferable and apparently gives rather sharper end-results in some cases. It avoids any source of error which may be due to the natural amboceptor for sheep blood which some specimens of human serum contain in excess. On the other hand, the use of an ox-rabbit haemolysin and ox-corpuscles occasionally gives rise to a difficulty in the technique due to the marked lytic action which some examples of guinea-pig serum possess for ox corpuscles. On

this account, a control must always be put up when carrying out the titration, and if the guinea-pig serum alone shows any lytic action, it ought to be discarded and another complement used. The amboceptor was invariably titrated each time before use and a quantity equal to  $2\frac{1}{2}$  times the minimal haemolytic dose employed for each tube.

(3) *Blood*. The blood was collected at the slaughter-house and defibrinated by shaking. A 5% suspension of the washed blood in salt solution was employed.

(4) *Complement*. The complement used was the serum of the guinea-pig, obtained by collecting the blood on the previous evening and allowing clotting to take place. The containing-vessel was then placed overnight in the ice-chest, and the serum pipetted off immediately before use. By collecting the blood in this way a larger amount of serum is obtained than by defibrinating the blood and collecting the serum by centrifuging, and also the serum is much freer from haemoglobin staining. There is apparently no diminution in the complementing activity of the serum due to the keeping of the blood overnight. The complement must invariably be titrated before use and its titre noted (see later under "Antigen").

(5) *Antigen*. This is the most important factor in the test and on its suitability the whole value of the reaction depends. The antigen which has been used exclusively in this work, and the one which has given better results than any of those with which it has been compared, has been a fresh emulsion of living tubercle bacilli in salt solution. It is made up in the following manner:

A young vigorously growing culture of tubercle bacilli on a glycerine-egg medium is scraped off the surface of the medium, care being taken not to remove any of the medium along with the culture, and carefully emulsified in 0.85% salt solution by grinding in a ball and socket mortar, and made up to a strength of 1.500, the bacilli being weighed moist. This emulsion is made up immediately before use and is not sterilised in any way either by heat or by the addition of chemicals. It is important that this emulsion be made freshly each time as its keeping qualities are doubtful, and in comparative experiments carried out with a fresh and an old emulsion on the same sera, at the same time, with the same complement, better and sharper results have been obtained with the fresh emulsion. This point is dealt with more fully in the second part of this paper.

The antigen made in this way must be carefully titrated before

use. The customary method of carrying out such a titration is to take a fixed amount of complement, generally two minimal lytic doses and to titrate diminishing amounts of the antigen with this, as shown in the following table:

Antigen	Complement	Salt Sol.	Sensitized Blood	Salt Sol.
0.5	2 M.L.D.	Sufficient to make	0.5	To make a total
0.45	„	1.5 c.cm. in each	„	bulk of 2.5 c.cm.
0.4	„	tube	„	in each tube.
0.35	„		„	
etc.	„		„	

The double vertical lines indicate times in the incubator (see later).

This method of titration gives, as a rule, excellent results and allows a suitable dose of antigen, say, half the titre, to be selected for the serum examinations. On occasions, however, the “Eigenhemmung” is so marked that one is forced to use only a very minute amount of antigen in the principal experiment, in order to avoid the unspecific absorption of complement and consequent “summation” effects, and these minute doses of antigen are not sufficient to bring about specific fixation in the presence of very small amounts of antibody.

This phenomenon (of “Eigenhemmung”) which may occur even when the same culture is used, which on a previous occasion did not give rise to any “Eigenhemmung,” was for a long time a source of difficulty, and it was only when the same antigen was titrated with several complements that it was recognised that the cause was to be sought for, not in the antigen, but in the guinea-pig serum. This difference in guinea-pig sera is very important and is not shown at all in the simple titration of the complement. A serum may be very potent in its complementing power and yet have such a capacity for being absorbed by an emulsion of tubercle bacilli, that it is almost useless in complement fixation work in tuberculosis.

This absorption of the complement is usually ascribed to some property in the antigen and is known as “Eigenhemmung.” In order to avoid this undesirable “Eigenhemmung,” and at the same time to permit the use of a large dose of antigen, it is necessary to carry out the titration of the complement, both alone, and in the presence of that amount of antigen which will be used in the serum examinations. When this is done, the total amount of complement absorbed by the fixed dose of antigen is at once shown, and it is not necessary to titrate the antigen separately. This is the method which has been employed throughout this work, and the titration has been carried out in one of two ways, viz.



A. *Titration of the Complement in the presence of the Antigen.*

Complement	Antigen	Salt Sol.	Amboceptor	Blood	Salt Sol.
0.05	0.5	To make	2½ M.L.D.	0.5	To make
0.045	..	1.5 c.cm.	..	..	2.5 c.cm.
0.04	..	in	..	..	in
0.035	..	each tube	..	..	each tube
etc.					

The smallest amount of complement which brings about complete lysis is read as the titre.

The suitable dose of complement for the serum examinations is arrived at by taking the sum of the titre of the complement alone and of the titre of the complement in the presence of the antigen. In this way we know that there will be two lytic doses of complement left in each tube after the unspecific absorption of the complement is complete.

B. *Titration of the Complement in the presence of Antigen and Normal Serum.*

Complement	Antigen	Normal Serum	Salt Sol.	Amboceptor	Blood	Salt Sol.
0.05	0.5	0.1	To make	2½ M.L.D.	0.5	To make
0.045	..	..	1.5 c.cm.	..	..	2.5 c.cm.
0.04	..	..	in	..	..	in
0.035	..	..	each tube	..	..	each tube
etc.						

The normal serum used in the titration must be inactivated by heating at 56° for 30 minutes.

Method B is probably the better of the two, and it allows the correct dose of complement to be read at once. It is essential, however, that a "pool" of normal sera should be used in the titration rather than a single serum. This is necessary owing to the differences in normal sera, some increasing the amount of "Eigenhemmung," others definitely diminishing it.

*Time.* The time allowed for fixation is important, and in this work I have found that 2 or 2½ hours give much better results than the usual one hour. The results seem to improve from 1 to 3 hours, but after this time there may be some falling off in the activity of the complement. Probably the best results are given when 2½ hours in the air incubator at 37° are allowed. It is, of course, essential that similar times and temperatures should be employed in the serum examination as in the titrations.

*Temperature.* Opinions differ somewhat as to the most suitable temperature for the occurrence of fixation in all complement-fixation

work. Some observers contend that the reaction takes place best at a temperature of  $37^{\circ}$  in a water-bath, whilst other workers maintain that the temperature of the ice-chest is to be preferred. Fildes and McIntosh in their work on the Wassermann reaction have, however, found that the air-incubator is, if anything, superior to both of the above methods and it is their procedure which has been employed throughout this work. After the fixation has occurred, the sensitized blood is added and the tubes returned to the incubator for one hour. At the end of this time the tubes are placed in the ice-chest and the final results read the following morning. All the usual controls must be included in every series of cases.

### RESULTS.

In reading the results obtained by this method in cases of pulmonary tuberculosis, no attention has been paid to small amounts of undissolved cells. Only those cases which gave complete or almost complete fixation have been considered positive. (By "almost complete fixation" is meant that there is only a slight tinting of the fluid whilst the great mass of the red cells remains undissolved.) By adopting this standard it is probable that a number of positive cases have been missed, but this has not been considered very important until the value of the reaction has been more thoroughly worked out.

#### *I. Results in cases of pulmonary tuberculosis with tubercle bacilli in the sputum.*

No. of cases	Complement fixation positive	Complement fixation negative
Stage I. 109	98 (=89.9 %)	11
„ II. 318	291 (=91.5 %)	27
„ III. 141	117 (=82.9 %)	24
Totals 568	506 (=89.08 %)	62

There is, apparently, a falling off in the percentage of positive results in the advanced cases (Stage III), but the numbers are too small to draw any conclusions on this point.

#### *II. Results in apparently healthy persons.*

The serum derived from 45 apparently normal individuals was examined on 204 occasions with invariably negative results.

### III. *Results in cases suspected of pulmonary tuberculosis but without tubercle bacilli in the sputum.*

(a) Diagnosis of tuberculosis confirmed by the opsonic index or by subcutaneous injections of tuberculin, or by both.

No. of cases	Complement fixation reaction	
	Positive	Negative
20	15	5

(b) Diagnosis of tuberculosis negatived by the opsonic index or by subcutaneous injections of tuberculin, or by both.

No. of cases	Complement fixation reaction	
	Positive	Negative
63	1*	62

\* This case gave only incomplete fixation at first. Three days after the last injection of tuberculin (for diagnosis) the fixation result was completely negative, whilst 14 days later it had again become positive.

### IV. *Results in diseases other than pulmonary tuberculosis.*

Only a few cases have been examined, but all have given a negative result unless some other form of tuberculous disease was present. In many of the cases the diagnosis has been confirmed post-mortem.

Cases	Complement fixation reaction
Sarcoma of the lung	Negative
Carcinoma of the lung	..
Carcinoma of the stomach	..
Malta fever	..
Carcinoma of larynx	..
Infective endocarditis (two cases)	..
Carcinoma of liver	..
Stone in kidney (two cases)	..
Cystitis	..
Lupus of pharynx	Positive (complete)
Tuberculous peritonitis	.. ..

## PART II.

This part of the work is supplementary to that already described, and deals with some points in connection with the antigen, and the effects produced by variations in its mode of preparation.

### I. *Comparative values of different strains of tubercle bacilli as antigens.*

In estimating the values of the different strains it is necessary that the comparative experiments should be carried out at the same

time with the same complement, and that the emulsions should be of the same density.

In this work, eight strains of tubercle bacilli isolated directly from sputum (*T. humanus*) have been subjected to numerous comparative experiments, and I have not been able to discover any marked differences in the values of the different strains, provided that subcultures of approximately the same age and degree of moisture be employed.

On the other hand, the results obtained with avian bacilli have been much inferior, when compared with the human strains under similar conditions. One experiment, in which a number of tuberculous sera was examined with a fresh human and a fresh avian emulsion, is given in tabular form. The definite superiority of the human emulsion is clearly shown.

	Human bacilli antigen	Avian antigen
Case 1	++++	++
„ 2	++++	o
„ 3	++++	+++
„ 4	+++	o
„ 5	+++	++
„ 6	++++	+++
„ 7	++	o

(++++=complete fixation; o=complete lysis.)

## II. *Comparative values of different media in the growth of tubercle bacilli for antigens.*

In this investigation four varieties of media were employed:

- (1) Glycerine-egg solidified at 80°;
- (2) Glycerine-egg coagulated at 100°;
- (3) Serum;
- (4) Glycerine-potato.

Numerous experiments were carried out under identical conditions, but without being able to establish the definite superiority of any one of the media. On the whole the best results were given when the glycerine-egg medium solidified at 80° was employed. This was probably due to the fact that this medium allowed of a rapid growth which was readily emulsifiable, whereas the other media gave a slower rate of growth and the culture was more difficult to work up into an even emulsion.

### III. *Comparative values of young and old subcultures as antigens.*

In the experiments designed to investigate this point one strain of bacilli has been employed throughout, grown always on the same medium, viz., glycerine-egg. This strain was isolated directly from sputum by the antiformin method, and after very numerous subcultures could be depended upon to give a fairly abundant growth on the egg medium, in from five days to a week. Numerous sera were examined with antigens made up from cultures of this strain of varying ages, but no marked differences were noticed except when very old cultures (over 12 weeks) were employed. The older cultures seemed to be slightly less valuable, but the differences were so slight that they were probably due to the greater difficulty experienced in preparing an even emulsion from the dry wrinkled growth of the older cultures.

The greater value of the young culture is best seen in the examination of weakly binding sera, very strongly positive sera giving good results with either antigen as seen in the next table:

	Serum	Antigen from culture 78 days old	Antigen from culture 5 days old
I.	0.1	++	++++
	0.05	+	+++
	0.025	o	o
	0.0125	o	o
	0.006	o	o
II.	0.1	++++	++++
	0.05	++++	++++
	0.025	++++	++++
	0.0125	+++	++++
	0.006	+	+++

(++++=complete fixation.)

### IV. *Effect of age on the antigen.*

The question of the keeping qualities of these emulsions of tubercle bacilli is an important one owing to the difficulty of having a young vigorous subculture of tubercle bacilli always available, whereas the manipulations would be much simplified if a large quantity of an emulsion could be prepared and stored.

The experiments on this point have been carried out with one strain of tubercle bacilli, and the emulsions have always been stored in the ice-chest. Very little difficulty has been met with in keeping these emulsions sterile, but occasionally they have become contaminated with moulds. All such contaminated antigens have been discarded.



In considering the effects of age, it is best to divide the description of the results obtained into two parts.

(1) *Times less than one week.*

In these experiments the antigens were made up not only from the same strain, but also from the same culture tube. For this purpose a very large tube or Roux bottle of the egg medium was used and growth was allowed to continue until the surface of the medium was covered with a dense luxurious layer of growth. The necessary amount of culture was removed by a loop when required, and the antigen prepared and stored in the ice-chest. The culture tube, in the intervals, was sealed and returned to the incubator.

A number of sera were examined with these stored antigens but only very slight differences were noticed, when the length of storage was less than one week. Two of these experiments are shown in the next table:

		Antigen and length of storage					
	Serum	Fresh	1 day	3 days	4 days	6 days	7 days
I	0.1	++	++	++	++	++	++
	0.05	+	+	+	+	o	o
	0.025	o	o	o	o	o	o
	0.0125	o	o	o	o	o	o
	0.006	o	o	o	o	o	o
II.	0.1	++++	++++	++++	++++	++++	++++
	0.05	++++	++++	++++	++++	++++	++++
	0.025	++++	++++	++++	++++	++++	++++
	0.0125	+++	+++	+++	+++	+++	+++
	0.006	+	o	o	o	o	o

(++++=complete fixation.)

(2) *Times longer than one week.*

In these experiments several strains of bacilli were employed for comparison, and the antigens were always stored in the ice-chest.

In comparing the values of these stored antigens with a freshly made one, both the serum of patients suffering from pulmonary tuberculosis and the Tuberkulose-Serum, Hoechst, have been employed. Quantitative examinations have been carried out in many of these cases.

Almost invariably better results have been obtained when the freshly prepared antigen was used, as will be seen from the following tables:

A. *Serum from cases of pulmonary tuberculosis.*

Case	With fresh antigen	With antigen stored for 20 days
1	+	o
2	+	o
3	+	+
4	+	+
5	+	+
Case	With fresh antigen	With antigen stored for 28 days
6	+	+
7	+	+
8	+	+
9	+	+
10	+	+
11	+	+

## Case 12. Examined quantitatively.

Serum	Fresh antigen	Antigen stored for 28 days
0.1	+	+
0.05	+	+
0.025	+	+
0.0125	+	+
0.006	o	o

B. *With the Tuberkulose-Serum, Hoechst.*

I.	Serum	Fresh antigen	Antigen stored for 12 days
	0.0125	+	+
	0.006	+	+
	0.003	+	+
	0.0015	+	+
	0.00075	+	+
II.	Serum	Fresh antigen	Antigen stored for 20 days
	0.0125	+	+
	0.006	+	+
	0.003	+	+
	0.0015	+	+
	0.00075	+	+
III.	Serum	Fresh antigen	Antigen stored for 28 days
	0.0125	+	+
	0.006	+	+
	0.003	+	+
	0.0015	+	+
	0.00075	+	+

( + + + + = complete fixation.)

The above tables show that much more constant results are obtained when a freshly made emulsion is used as antigen. The effects of storage for periods less than one week are as a rule not very marked,

but if longer periods are allowed the results become irregular and errors are quite likely to arise, especially in the case of weakly binding sera. The Tuberkulose-Serum, Hoechst, does not show this superiority of the fresh antigen so well, but this is probably due to the high potency of the serum, which can bring about fixation of complement in the presence of almost any tubercle antigen.

The above tables show that it is necessary, when using this antigen, to make up the emulsion immediately before use, and that it is advisable to use a young subculture of the tubercle bacillus rather than an old dry one.

V. *Effect of storage on the amount of "Eigenhemmung" possessed by an emulsion of tubercle bacilli.*

This question was investigated by titrating the antigen at different ages with a fixed amount of complement. Two multiples of the minimal lytic doses of each complement being employed and the antigen being kept in the ice-chest in the intervals. This method, however, gave very unreliable results, the amount of "Eigenhemmung" seeming to fluctuate in either direction irregularly, as will be seen by one of these experiments shown in the next table.

The explanation of this was at first difficult, but the irregularity is, I think, caused by differences in the complements employed, rather than by any changes in the anti-complementary power of the antigen as the result of age:

Amounts of antigen	Ages of the antigen			
	1 day	5 days	24 days	31 days
0.4	+	o	+	+
0.45	+	o	+	+
0.4	+	o	+	o
0.35	o	o	+	o
0.3	o	o	+	o
0.25	o	o	o	o
0.2	o	o	o	o

(+ + + + = complete inhibition of haemolysis; o = complete lysis.)

### PART III.

In this section, the effects of exercise and of tuberculin on the antibody content of the serum are described.

I. *Effects of exercise on the antibody content of the serum.*

This investigation was undertaken to discover whether any fluctuations in the amount of complement-fixing bodies took place as the

result of exercise, analogous to the fluctuations in the opsonic index. The results were, however, quite negative, the titre of the serum remaining unaffected in either direction, as is seen from the two examples given in the tables.

CASE 1. *Pulmonary tuberculosis.* T.B. +.

Serum	Before exercise	1 hour after	6 hours after	24 hours after
0.1	++++	++++	++++	++++
0.05	+++	+++	+++	+++

CASE 2. *Pulmonary tuberculosis.* T.B. +.

Serum	Before exercise	1 hour after	6 hours after	24 hours after
0.1	o	o	o	o
0.05	o	o	o	o

(++++=complete fixation; o=complete lysis.)

## II. *Effect of tuberculin.*

A number of experiments have been carried out to investigate this question, partly in normal, partly in tuberculous subjects.

In the majority of these cases the tuberculin had been given subcutaneously for diagnosis, but in a few cases of definite tuberculosis the reaction was carried out before and also at the end of a course of tuberculin treatment.

The results obtained have been very irregular, especially in those persons who received cumulative doses of old tuberculin for diagnosis. In all these cases the test was completely negative before the administration of the tuberculin, whereas afterwards some remained negative, others became positive temporarily. I have no explanation to offer for this change in the reaction, as most of these patients had no other evidence of tuberculosis.

A few of the results obtained are shown in the table:

Case	Result before tuberculin	Result after tuberculin
1	o	o
2	o	o
3	o	o
4	o	+++
5	+	++++
6	o	+
7	o	+
8	o	++
9	o	++
10	o	o
11	o	+++
12	o	+
13	o	o
14	o	o

(++++=complete fixation of complement.)

(o=complete lysis, *i.e.*, no fixation of complement.)

In the cases of definite pulmonary tuberculosis with tubercle bacilli in the sputum, the usual result of a long course of tuberculin treatment was to cause an increase in the amount of complement-fixing bodies in the serum, provided that these bodies had been present in fair amount before the treatment was commenced. On the other hand, in those cases which had given a negative result in spite of their tubercle bacilli, the tuberculin treatment did not seem to cause any development of these bodies in the few cases investigated.

#### SUMMARY.

1. A fresh emulsion of tubercle bacilli in salt solution when used as the antigen will give about 90 % of positive results in cases of pulmonary tuberculosis, whilst in healthy persons the result is negative.

2. In cases suspected of pulmonary tuberculosis, the results of this test conform closely to those given by a combination of the opsonic index test with injections of tuberculin, and so may be of value in diagnosis.

3. Diseases other than tuberculosis have always been negative, but only a small number of such cases have been examined.

4. An emulsion of tubercle bacilli when used as an antigen must be freshly prepared immediately before use, and should be made from a young subculture.

5. Owing to the differences in complements, they should always be titrated in the presence of the antigen, and probably also in the presence of both antigen and normal serum.

6. Exercise does not seem to affect the result of the reaction in any way, but a course of tuberculin treatment leads to an increase in the complement-fixing bodies of the serum in some cases.

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## THE SUPPLY OF NON-TUBERCULOUS DAIRY STOCK.

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THE object of this paper is to draw attention to the need of a further supply of sound dairy stock. Three main arguments in favour of this proposal have occupied our minds: (1) that the loss to the dairy industry through tuberculosis in cattle amounts to a considerable sum each year, (2) that there is serious loss in human lives due to bovine tuberculosis, (3) that, owing to their dearth in the country, there should be sufficient demand for sound dairy stock to place a farm devoted to the rearing of such cattle on a sound financial basis. The last part of this paper deals with a proposal to carry out this scheme.

The annual loss to the dairy industry through tuberculosis is undoubtedly considerable, although the loss to any individual farmer is very difficult to estimate. The following table is probably a very moderate estimate of the amount of this loss:

Cows and heifers, etc., 2 years and above in England and Wales in 1913	.. .. .	3,415,028
2 % of these being wasters	.. .. .	68,300
Wasting probably due to tuberculosis in at least one- third of cases	.. .. .	22,766
Average loss on each waster	.. .. .	£12
Total annual loss	.. .. .	£273,192

It would be possible to give a very much more accurate estimate if a birth and death register of dairy cattle were kept. When we

remember the extreme and ever-increasing value of the Registrar General's records of births and deaths for human beings, and the extraordinary influence these have had on modern legislation in producing a nation, longer lived and healthier than has ever been known in the past, it seems incredible that no attempt has been made to establish similar records for one of the most important industries of our country. Progress in the prevention of diseases of animals must always be hampered so long as there are no true records upon which to base procedure. A small fee to the veterinary surgeon would produce a record of constantly increasing value, which would bring home to us the true extent of our losses and direct our energies to the most pressing problems of the diagnosis and prevention of animal disease.

It was hoped that the Tuberculosis Order, which deals with tuberculosis of the udder, tuberculosis with emaciation, and cows giving tuberculous milk, that is to say, advanced cases only, would give a measure of the extent of patent tuberculosis. This result cannot yet be said to have been attained. Professor McFadyean has recently pointed out that the number of cows and heifers in milk or calf in Great Britain in 1913 was 2,695,391. During the twelve months, April 1913 and April 1914, notice was given under the Order of the intended slaughter of 6738 bovine animals, or 1 in 400 of the cows and heifers in milk or in calf. These figures indicate that the provisions of the Tuberculosis Order have been evaded by the great majority of owners, and that probably not more than 25 % of the clinically tuberculous animals have been reported. It is probable that Professor McFadyean's estimate is correct.

We have ourselves made an attempt to estimate the possible number of cows supplying tuberculous milk for human consumption in England and Wales. Our calculation is based on the statistics obtained by the examination of samples of mixed milks in London and Manchester for tubercle bacilli. Of 15,008 samples, 9.3 % showed *Bacillus tuberculosis*. These results have been obtained in large cities, where the routine examination of milk for tubercle has been carried out for many years. This is known to the farmers supplying these cities. In districts where less strenuous efforts are made a very much larger proportion of tubercle bacilli is found in mixed milks, thus Mitchell, for example, found 20 % in milks bought in Edinburgh dairies. Similar results have been obtained in country districts where supervision was not stringent. If we take 10 % as an average this is certainly not too high an estimate. On an average a milk churn contains the milk of 15 cows, and therefore

at least one cow in 150 is excreting tubercle bacilli in its milk. The total number of cows and heifers in milk and in calf in England and Wales in 1913 was fully 2,250,000. Of this total 1,750,000 may be assumed to be concerned in the supply of milk and milk products for human consumption. On the basis that 1 cow in 150 produces tuberculous milk, a total of 11,666 cows and heifers in a dangerous state of tuberculosis is obtained. It is interesting to note that a similar result is obtained by a different method. It was assumed on an earlier page that 2 % of the cattle over 2 years old in England and Wales were wasters, and that one-third of this 2 % suffered from "tuberculosis." Two per cent. of the 1,750,000 dairy stock is 35,000 and one-third of this number is 11,666. We are aware that these calculations are subject to criticism, as for example, that the same cow may be infecting the milk of two dairies, that some of the samples may have contained tubercle bacilli of human origin, that some of the samples taken may have been repeat specimens, etc. It is none the less interesting that the two methods adopted give results so closely corresponding one to another, and it cannot be denied that they are appreciably lower than those usually stated, yet represent a very definite loss to the dairy industry. It must also be remembered that the Tuberculosis Order was designed to remove from herds the most extreme cases of infection, it does not affect the vast majority of the cases of tuberculosis.

From all the evidence at present available, it may be taken that about 30 % of the cattle in this country are affected with tuberculosis and that of the total cattle not more than 2 % will be in such an advanced state of the disease as to be affected by the Tuberculosis Order. What then is to become of the remaining 28 %? Some will pass on to advanced tuberculosis and eventually come under the Order, having in the interval infected other animals in the herd. Some, exactly what proportion it is impossible to say, will remain chronic sources of infection; since the work of Griffith, Klose, Rosenberger, Phillip and Porter, Moore Alexander, etc., has shown quite clearly that, whatever the situation of the tubercle bacilli in the body may be, they may escape from the animal in the various excrements.

Schroeder writes: "Tuberculosis of cattle, as of persons, may be acute or rapidly progressive and run its ordinary course quickly from infection to death. This is rare. As a rule it is insidious, chronic and slowly progressive, and the bodies of its victims are able to adapt or adjust themselves to the gradually increasing, destructive changes it causes until quite extensive harm has been done or vitally important

organs have been seriously involved. The result is that the disease may be present in the body for a long time without external manifestations of its existence. It may attack any part of the body singly and remain confined to it or it may attack several or many parts simultaneously or successively. Its favourite location in the bodies of cattle, as in those of persons, is the lung.... Tuberculosis is more effectually concealed in the bodies of cattle than in those of persons, and we all know how long a diagnosis with persons may remain in doubt...." Schroeder then proceeds to show that excessive coughing is very rare in cattle and occurs only during the last stage of pulmonary tuberculosis, that when cattle cough, they do not expectorate, but only raise the material which has accumulated in their air passages far enough into their mouths to be swallowed. "The tubercle bacilli that are expelled with the faeces per rectum have their origin in the lung more commonly than elsewhere. They are raised into the mouth and then swallowed and on their way through the intestinal tract become evenly mixed with the material that is ejected as faeces from the bowels; the result is that the large amount of faeces passed by cows, about 30 lbs. per day by a cow of average size, introduces an enormous amount of infectious material into their environment when they are affected with tuberculosis." Further, after giving numerous illustrations of cattle which were apparently in good health, yet were shedding tubercle bacilli in their excreta, he concludes that, "with few exceptions, the character of tuberculosis among cattle is that of an effectually concealed disease, the detection of which before it is well advanced and has done great harm is impossible through the agency of our own unaided powers of observation. Fortunately we have in tuberculin an excellent agent for detecting tuberculosis in cattle when all other means of diagnosis fail." It is impossible to say what will be the ultimate fate of an animal which reacts to tuberculin; the certain fact is that among these animals there are those which do not come under the provisions of the Tuberculosis Order, yet are a constant source of infection in the herds in which they exist, and are the cause of very considerable loss to the farmer.

We have shown that the loss to the dairy industry through bovine tuberculosis is considerable, we feel that we ought also to give some measure of the loss in human life, especially as lately some very thorough and interesting work has been published on this subject, and the longer the work continues, and the more carefully it is done, the greater is the conviction that, if tuberculosis is to be eradicated in man, bovine



tuberculosis cannot be neglected, especially as a disease of children. Twelve months ago we published a paper, in which we estimated that at least 4000 children a year died of this disease, and that there are always 40,000 children suffering from it. The annual loss to the country in extra expense due to this illness must be, therefore, not less than about £400,000. About the same time, Délépine published a paper in which he showed that in Manchester, where very special efforts have been made to secure a tubercle-free milk supply, the death rate from those forms of tuberculosis due to bovine infection had decreased more rapidly than those forms due to human infection. The results of recent investigations confirm these statements. Thus Eastwood, F. Griffith and A. Stanley Griffith investigated the incidence of tuberculous infection in a series of children (hospital cases), dying from *all causes* between the ages of 2 and 10 years, with the following results:

Cases investigated .. .. .	195
Cases showing tubercle bacilli .. .. .	118
Cases in which tubercle bacilli could not be grown, their nature (human or bovine) could therefore not be determined ..	20
Cases in which the bacilli could be grown .. .. .	98
Cases of bovine tuberculosis .. .. .	17
Cases of human tuberculosis .. .. .	80
Case showing both bovine and human types of bacilli .. ..	1

These figures are of great interest. They show, in the first place, that of a very large proportion of these children dying from *all causes* about 60 % suffered from tuberculous infection, and that in those cases in which it was possible to obtain growths of the bacillus, rather more than 17 % were of the bovine type.

Fraser and Mitchell in Edinburgh have investigated this question from another point of view. Fraser examined tuberculous material from 40 children, aged 0 to 5 years who were suffering from tuberculosis of the bones and joints, and found the infection to be bovine in 28 cases, 12 being infected by the human type of bacillus. He also examined tuberculous material from 23 children aged 5 to 16. In this series the bovine type of bacillus was the cause of infection in 9 cases. Mitchell showed that, of 80 cases of tuberculous infection of the glands of the neck in children, 71 were caused by the bovine type of bacillus. In America similar results have been obtained. Of 95 cases of tuberculous infection of the glands of the neck, 51 were due to the human type and 44 to the bovine. Again, in cases in which the infection was in the abdomen, it was found that of 86 cases 34 were infected by the



human type, 52 by the bovine type of bacillus. All these statistics concern children under 16, that is to say, the chief milk drinkers. It is not claimed that bovine tuberculosis causes more than a fraction of the total death rate from tuberculosis, but it is a fraction, a very definite fraction, which causes the loss of valuable lives and is a constant source of expense to the country.

Since the loss to the dairy industry is considerable, and the loss in human lives is not to be neglected, an increase in the supply of sound cattle should appeal, both to the Agricultural and Public Health Authorities. It should also appeal to the financier, for there is no doubt, that at the present time, there is a dearth of dairy stock. The Board of Agriculture and Fisheries reports (Cd. 7325) that for the year 1913 "the number of cattle of all classes was 5,716,944, being 124,776 (2.1 %) fewer than in 1912, and 197,303 fewer than in 1911, when the stock of cattle was the largest yet returned. The total in 1913 was the lowest returned since 1904.

The net reduction of 124,776 cattle was made up as follows: cows and heifers in milk, decrease 141,458; cows and heifers in calf, but not in milk, increase of 57,942; other cattle, 2 years and above, increase of 38,430; other cattle, 1 year and under 2, decrease of 78,882; and other cattle under 1 year, decrease of 808." This decrease in the dairy stock of the country together with the facts previously stated showing the loss from Tuberculosis both in cattle and man makes it evident that it is highly desirable to take further steps to lessen the prevalence of this disease. The question therefore is, how can this best be done so far as cattle are concerned? To answer this question three propositions must be considered:

- A. The method of diagnosis must be as accurate as possible.
- B. There must be complete separation of infected from non-infected animals, and the thorough disinfection of cow byres, etc.
- C. Possible fresh sources of infection must never be re-introduced into a tubercle free herd.

A. No one will deny that it is quite impossible to diagnose tuberculosis in its early stages by clinical methods alone. There remain, however, other methods of diagnosis, of which the tuberculin test, when properly carried out, gives results the accuracy of which is not obtained in any other way, *e.g.* the Royal Commission on Tuberculosis tested 1000 healthy Jersey calves and obtained a reaction on four occasions, an error of 0.4 %. Délépine, in the Ford Bank experiment, in which

comparatively few cattle were tested, found that "tuberculin applied to cattle not exceeding 8 or 9 years of age may give absolutely reliable indications at the first application of the test in 98 % of the animals tested, and that by repeating the test after an interval of 2 or 3 months the margin of error may be reduced to nil by careful consideration of the state of the animal." Noeard says that "everyone admits the exactitude and wonderful accuracy of tuberculin as a means of diagnosis." Unfortunately everybody has not yet admitted it, and therefore it is necessary to lay some stress upon this point. Where the accuracy of the test is accepted and the farmer has had it applied to his cattle, it is most essential that he should carry out *B*, the separation of the reactors from the non-reactors, and the disinfection of all cow byres, etc. It is possible for an enlightened farmer possessing the necessary buildings to carry out these conditions, fattening and killing his reactors, and cleansing and disinfecting his buildings, and many would be prepared to do it, in spite of the fact that there must be loss upon the animals which are discarded and some dislocation of their milk contracts, if they could only be certain that it was possible to replace their discarded cattle by thoroughly sound stock. At the present time there are only two ways by which a farmer can replace his stock: (1) he must purchase in the open market without any reference to the tuberculin test, or (2) purchase them subject to the test. These methods are unsatisfactory. One very large dairy farmer informed us that 50 % of the cattle purchased by him under the second method reacted after being three months on the farm, while the purchase of cattle by the first method with subsequent testing is almost certain to involve serious financial loss.

Many farmers who are willing to have their cows tested and possess satisfactory buildings for isolation have hesitated because of this difficulty of replacement of stock, and under the present conditions they cannot be blamed. It appears that at least two possible ways might be found out of the difficulty; that the farmers should combine together and use one farm as an isolation station. It is very improbable that such a scheme is likely to be adopted in this country at the present time. That centres should be established for the production of warranted stock, which should be sold to farmers prepared to take the necessary steps to clear their herds and to keep them clear.

In any scheme which might be offered to fulfil this object it must be remembered that it is essential to be able to produce a sufficient supply of milch cows, and therefore, it does not seem advisable to adopt

a method which involves a breeding herd, as there would be danger of excess of bull calves in some years. The plan proposed is that calves (mostly heifers) should be purchased within a very short period of birth, and should be reared under the best conditions, all those which reacted to tuberculin being eliminated and the remainder sold as down-calving heifers.

This plan offers a very excellent chance of success, especially if the precautions mentioned in the later part of this paper are adopted, since tuberculosis is extremely rare among young calves. The following statistics are sufficient to establish this fact:

					Slaughtered	Tuberculous
Saxony 1895						
Cows	..	..	..	..	39,495	32.49 %
Bulls	..	..	..	..	18,149	20.99 %
Calves	..	..	..	..	201,643	0.24 %
Kiel 1895-1898						
Calves less than one week old				..	21,858	0.63 %
Flensburg 1899-1906						
Calves	..	..	..	..	24,828	0.72 %
Schwerin 1893-1903						
Calves	..	..	..	..	43,449	0.21 %

The idea of a farm specially adapted and used for the rearing of calves is therefore most likely to meet the requirements of the situation, and the large number of progressive farmers who are at present considering the advisability of having their cows tested, with a view to obtaining a "free" herd, makes it abundantly clear that were heifers from such a farm available there would be no lack of purchasers. The most important points of such a scheme are discussed below.

*Type of farm required.* The farm on which the calves were to be reared would be required to be carefully selected and would necessarily be one naturally well fitted for the purpose, *i.e.* one with a fair acreage of grass land of moderate quality, fields with a certain amount of shelter and fairly dry buildings on a healthy site, and a plentiful supply of good water. A small acreage of arable land (say one-fifth to one-quarter of the whole) would be desirable in order to grow a supply of roots and hay for home consumption, while of the grain crops oats would be generally preferable, as both the straw and the grain would be useful, the value of crushed oats as a food for young calves having recently been convincingly proved by the Royal Agricultural Society's experiments at Woburn.

*Management of the calves.* The calves would be taken from their

dams when three days to a week old and transported to the farm, there to be housed and fed with all possible care. When old enough they would be tested, and all reacting to the test would be slaughtered. It is obviously much cheaper to slaughter calves a few weeks old than to rear them to maturity and then test, isolate and dispose of them with all the associated risk of infection and death.

Non-reacting calves would be kept by themselves and fed most carefully, using either milk which was known to be free from tubercle, pasteurized milk, or some of the numerous milk equivalents which have been tested and proved satisfactory. They would be reared generally under perfectly hygienic conditions, the object being to produce heifers which have not been in any way pampered or made too fat, and which are likely to turn out satisfactory dairy cows with thoroughly sound constitutions. Any animals showing signs of illness would be at once isolated. The second tuberculin test would be carried out when the animals were 12 months old, and finally before being put to service, the reactors, if any, being isolated and fattened.

Those showing no reaction at this time would be served by a non-reacting bull and would be ready for sale as down-calving heifers when  $2\frac{1}{2}$  to 3 years old. The bull used should be a pedigree or half-pedigreed bull and descended from a milking family with good milk records.

*Purchase of calves.* For such a scheme as the above to be successful care would have to be exercised in the selection of the heifer calves. Many dairy farmers who at present do not rear any calves because of the value of their land or the lack of accommodation have cows of excellent type, but use a bull of very inferior quality. While the calves from such a bull are likely to be far from satisfactory for the purpose in view, those from the same cow by a pedigree or half-pedigreed bull would be very desirable animals, and valuable additions to the dairy stock of the country. Prices on a slightly higher scale might be paid where the calves were by a pedigree bull or where the farmer kept milk records and information on these points was obtained when the calf was bought. In the districts where Milk Record Societies are in existence it should be possible to arrange for a supply of calves from cows with known records, and in this case a few bull calves from exceptionally good cows—good in type and substance as well as in milk yields—might also be purchased and reared to be sold later as non-reacting dairy bulls of definite milking ancestry.

Now that the value of milk records in the dairy industry is being more generally recognised, there should be a keen demand for well-



grown heifers from cows with known records and in-calf to a good dairy bull.

At the beginning of any such scheme it would probably be impossible to consume all the home-grown fodder, or to make any quantity of dung with simply a stock of calves, but this difficulty could be largely overcome during the first year or two by the purchase of a number of heifers fit for service. These would be tested, all reactors isolated and fattened, and the remainder served and sold as down-calvers. If Irish heifers were bought it is probable that but a small percentage would be found to react to the test.

By the third year there should be a full complement of home-reared stock, and from that time onward it should be possible to say that all animals on the farm were free from tuberculosis.

*Buildings.* On most farms which would be at all suitable for the purpose of this scheme it is highly probable that there would be buildings which could be adapted to the housing of yearlings and bulls besides the necessary stables and food store, but it might be necessary to erect, or to alter materially a building which would make a satisfactory calf shed, and another which could be used as an isolation shed.

The calf shed would be for the accommodation of calves which had passed the test until they were beyond the stage for requiring liquid food. Such a shed must be warm, well ventilated and lighted, and capable of thorough disinfection; a concrete floor would be desirable with the walls of smooth concrete for three or four feet from the ground. Small pens of 20 to 25 square feet should be provided for the youngest calves to keep them from sucking each other; the partitions should be raised from the floor level and should fit into sockets in posts set in the concrete, but be capable of easy removal for thorough disinfection; also feeding would be greatly simplified if the partitions were made of open wood-work with movable spars. If it were necessary to economise space the calves could be tied by the neck in a part of the shed fitted for the purpose.

For calves which had passed the stage of liquid food, pens could be used large enough to accommodate four to six calves, with a feeding trough along one side, but such a trough should be fitted with ties or stanchions so that each calf may get its share of food. One large shed could be arranged with small pens for single calves, and larger pens for groups of calves, while if a new building had to be erected, the width, slope of floor, position of door, etc., could be so planned as to make it easily convertible into a building suitable for some other purpose.



The isolation shed would need to have a sound floor, smooth walls, and be easily capable of thorough disinfection.

A small separate compartment for the food store, convenient to the calf shed, should be reserved for the calves' food and fitted with a boiler to supply hot water for the preparation of the food, washing of pails, etc.

*Supervision.* It is essential to the success of any such scheme that the farm should be conducted in such a manner as to warrant implicit confidence being placed in the quality of the stock it produces, and their freedom from tuberculosis.

There are several cases on record and many others known to farmers throughout the country, where unsuccessful attempts have been made to get "free" herds, and when these cases are enquired into it is usually found that some important details have not been attended to—isolation has not been properly or effectively carried out, disinfection has been irregular and inefficient, quarantine periods have not been properly observed, etc. Where a disease is so widely distributed and of so insidious a nature as tuberculosis, it is of supreme importance that all these points receive careful attention. When it can be once said that a farm or herd is entirely free from the disease, no effort should be spared and no precaution neglected to prevent its re-appearance, and on such a farm as that suggested above the adoption of the most improved methods to obtain this end would need to be insisted upon. It would be equally necessary to be sure that all these methods were competently carried out.

It would be desirable that accurate records be kept of the history of all animals, so that the fullest information as regards their ancestry and health might be at the disposal, not only of the owner, but also of the prospective purchaser. The purchaser of any animal from such a farm should receive a certificate giving full particulars, with dates, etc. of the tests to which it had been subjected, while the animal itself might be tattooed in the ear, or otherwise marked in such a way as to make identification always possible. If the subsequent performance of heifers sold from the farm were recorded much information would be obtained regarding the effect of the sire in the inheritance of milk production.

While it may readily be granted that the establishment of such a farm is desirable, or necessary, before substantial progress can be made in the eradication of tuberculosis from our non-pedigreed dairy herds, the actual launching of any such scheme will undoubtedly require

careful thought and the co-operation of the practical farmer and breeder with the veterinarian and bacteriologist.

The suggestion has already been made that the local authorities, by establishing and maintaining clean herds for the supply of milk to public institutions would supply valuable object lessons<sup>1</sup>, and several city councils have already moved in this direction. Also in the Remkold district in Denmark an association of farmers has been formed with the intention that members shall have free herds, so that the district may become a centre for the breeding and distribution of sound stock<sup>2</sup>.

In this country some progress has been made in the same direction by the farmers in Wensleydale who are members of the Wensleydale Pure Milk Society, Limited, but in this case it is specially stated that the chief disadvantage the farmers suffer from is the fact that they are continually buying and selling cattle, and thus frequently running the risk of introducing fresh sources of infection.

There are several sources from which the initiative and capital required to commence such farms might come. Where the farmers are already formed into societies dealing with stock breeding or milk records a move in the direction of the tenancy and management of a farm for the rearing of their own calves would not be impossible; it is but a step further from the co-operative societies engaged in the purchase of manures and foods, and the growing of farm seeds.

Perhaps the conditions of dairy farming which lend themselves most readily to the establishment of such a farm is where a farmer or landlord works several farms and keeps a large number of cows; one of these farms could be equipped and used as a rearing farm for the heifer calves, and the herds of cows at the other farms could be tested and brought up to the "free" standard one at a time. A farm of 150 acres should keep 100-120 stock of all ages and have 30-40 in-calf heifers per annum after the second year. Under such conditions the farms would ultimately have only home-bred "free" stock and be entirely independent of outside purchases except for occasional bulls.

A third possible method of commencement is the taking of a farm by one or more individuals and the management of it on the system outlined above, with whatever modifications might be necessary to suit local conditions. Careful calculations have been made as regards the capital required and the probable returns, and the prospects of such a

<sup>1</sup> Dr Eastwood's Report to the *Local Gov't. Board*, p. 26.

<sup>2</sup> *Journal of the Board of Agriculture* for July, 1913.

venture appear to be distinctly bright simply as a business proposition. Dairy farmers in the South of England will agree that for some time back the prices made by good class heifers have been more satisfactory to the seller than to the buyer, and that any change in the situation is unlikely to take place for some years to come.

As is usually the case on taking a new farm, no profit would be made in the first, and perhaps the second year, but afterwards a satisfactory return should be obtained on the capital invested, while a distinct step forward would have been made towards the complete eradication of tuberculosis.

It has already been pointed out that the Tuberculosis Order of the Board of Agriculture and Fisheries, while stamping out of our herds the most flagrant sources of infection, still leaves in those herds animals infected with tuberculosis, some of which will themselves become active agents in spreading infection both to other members of the herd and also to the milk supply. The present Order can, therefore, have no finality. The recently introduced Live Stock Scheme of the same Board gives facilities for the small farmer to improve his stock, but does not provide him with sound cattle. Could not, therefore, another scheme be evolved which would bridge the gap and supply the farmer, not only with better stock, but also with sound stock?

Rearing farms carried out on the lines here suggested would appear to accomplish this object, and if taken up by responsible authorities, would be of the greatest assistance in the improvement of the health and quality of dairy cattle. Moreover, such rearing farms could be made financially successful. The proposal seems worthy of the consideration of county and other authorities, and its value would be still further enhanced if a system of inspection and registration of rearing farms and their stock were devised. The work accomplished by the Tuberculosis Order would be thus extended, and dairy farmers would be encouraged to have their herds tested if they knew that they could replace the reactors by sound stock. It would also increase the number of calves reared and lead to a better supply of milk-cows of known breeding, good type and sound constitutions. Finally, in the course of time, small areas would arise in which all stock would be guaranteed non-tuberculous. Such areas would be models of management and centres for the breeding and distribution of healthy cattle, an advance, of incalculable advantage not only to the individual, but also to the State, with a far-reaching influence in reducing the present enormous mortality from tuberculosis.

## CONCERNING INOCULATION AGAINST PLAGUE AND PNEUMONIA AND THE EXPERIMENTAL STUDY OF CURATIVE METHODS.

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THE present notes have been written in connection with official correspondence regarding the prophylactic inoculation against plague and the study of this and other health problems in India.

### PART I.

#### *On prophylactic Inoculation against Plague and Pneumonia.*

The method of anti-plague inoculation was introduced in January, 1897; and in the year following its effects were subjected to an enquiry by a Government commission known as the Indian Plague Commission, 1898-99.

The report on that enquiry was published in 1900-01, and therein the Commission formulated views different from mine on a variety of subjects, some of considerable importance. Among the members employed on the enquiry were professors of Medical Colleges and civil officials serving in high posts with the Governments of India, Bombay and the Punjab, and their findings have been and are, very properly, viewed as a guide in matters affecting the plague inoculation. It is, therefore, essential that such of their pronouncements as were due to incomplete information available at the time or to misunderstanding should be rectified when the subjects become clear.

Two of the members of the Commission, Professor, now Sir Ahnroth E. Wright and Dr Armand Ruffer, had, sometime previously to their appointment on the enquiry, been initiated by me in the principles and



methods of preventive inoculation against cholera<sup>1</sup>; and, at a subsequent date, in 1896, I had started one of these members, Prof. Wright, upon the work of preventive inoculation against typhoid fever<sup>2</sup>. It will appear from a statement by Sir A. E. Wright, quoted lower down, that it was largely his views—but, no doubt, also Dr Ruffer's—that guided the Plague Commission in their conclusions regarding certain aspects of anti-plague inoculation. Latterly, Sir A. E. Wright, together with Drs W. Parry Morgan, L. Colebrook and R. W. Dodgson, have been employed, for some two years, in investigating the pneumonia among the Rand Mine labourers in South Africa. The account of their findings throws light on certain subjects dealt with by the Plague Commission of 1898-99; and in view of the part which Sir A. E. Wright had taken in the framing of the Commission's conclusions, the result of the above authors' studies may be taken as supplementing the materials for a right understanding of the questions involved.

Accordingly, in the notes which follow, the points of divergence between the Commission of 1898-99 and myself which have come within the scope of the enquiry in South Africa have been gathered together and reviewed in consecutive order. Part of the matter concerns fundamental principles. I need hardly add that, in the years that have intervened, I continued to study carefully all the subjects on which the Commission of 1898-99 or any other experts had dissented from me; the present review is, however, limited to the items specified, on the grounds already explained.

The text of this note is divided into sections according to the several subjects dealt with; and each section is arranged, for facility of reference, under three heads, viz., as follows:

Sub-division *A* refers to the information and views placed by me before the Plague Commission of 1898-99 and reported in the

<sup>1</sup> *Vide* A. E. Wright and Surgeon-Captain D. Bruce, "On Haffkine's method of vaccination against Asiatic Cholera," *British Medical Journal*, 4th February, 1893, pp. 227-231; and W. M. Haffkine, "Injections against cholera," *The Lancet*, 11th February, 1893, pp. 316-318. The latter paper was read by Dr Ruffer at a meeting in the Conjoint Research Laboratories of the Royal Colleges of Physicians of London and Surgeons of England, where, in co-operation with him and with Prof. Sims Woodhead and Dr Cartwright Wood, I had demonstrated the principal experiments connected with the subject. The operations performed with Prof. Wright and his then assistant, Surgeon-Captain, now Surgeon-General Sir David Bruce and the Surgeons on probation at the Army Medical School in Netley were, however, of a more elaborate and detailed character, as may be seen from the first of the publications just mentioned.

<sup>2</sup> A. E. Wright and Surgeon-Major (now Colonel Sir David) Semple, "On vaccination against typhoid fever," *British Medical Journal*, 30th January, 1897, pp. 256-259.



"Minutes of Evidence," vols. I and III of their *Report* ("Indian Plague Commission, 1898-99." Government Publication, printed for Her Majesty's Stationery Office, Eyre and Spottiswoode, London, 1900);

Sub-division *B* contains the Commission's analysis and conclusion regarding the subject concerned, and is quoted from their *Report*, vol. V, issued in 1901; and

Sub-division *C* gives the results arrived at by Sir Almroth E. Wright and Drs Morgan, Colebrook and Dodgson, in the course of their present enquiry, as recorded in the "Report to the Witwatersrand Native Labour Association on the Results of an Inquiry into the Causation, Prophylaxis, and Treatment of the Pneumonia which affects the Native Labourers, and in particular the Tropical Native Labourers in the Rand Mines.—Observations on Prophylactic Inoculation against Pneumococcus Infections, and on the Results which have been achieved by it. By Sir Almroth E. Wright, M.D., F.R.S., in conjunction with W. Parry Morgan, M.B. Cantab., L. Colebrook, M.B. Lond., and R. W. Dodgson, M.D. Lond." *The Lancet*, 3rd and 10th January, 1914, pp. 1-10 and 87-95.

## SECTION I.

The subject of this section has been touched upon in my "Epidemiological Notes," dated Calcutta, October, 1911, in which I had occasion to refer to inoculation against plague and remarked, with regard to the Indian Plague Commission of 1898-99, as follows: "A general source of error was created by their rejection of the thesis established by the work of anti-plague inoculation, viz. that *the treatment was effective in people who were already harbouring infection in their system*, and that it was thus possible to influence an outbreak of plague in a few hours."

The statements which I had made to the Commission regarding this thesis are contained in the following quotations.

### A.

*Passages in the evidence given to the Indian Plague Commission, on their 1st day's sitting, the 29th November, 1898, concerning the effect of inoculation in the incubation stage of plague.* ("Indian Plague Commission, 1898-99. Minutes of Evidence," vol. I, pp. 5 and 6.)

(Section 34): "From the next morning (after the operations performed in the Byculla House of Correction, Bombay, where 154 prisoners

out of a total of 337 had been inoculated) a difference appeared between the inoculated group and the non-inoculated."

(Section 35): "The difference in their relation to the disease appeared from the next morning."

"From the next morning after the inoculations there were altogether twelve cases of plague, of which six proved fatal, amongst the non-inoculated; and two cases, both of whom recovered, among the inoculated." (Section 36): "The prophylactic was powerless to repress the symptoms of plague already started, or which developed within a few hours after inoculation. (Section 37): (*The President.*)—In that quantity?—Yes, in the quantity used. This conclusion was drawn from the fact that the only prisoners who did not seem to have benefited by the inoculation were the one who had a bubo at the time he came to be inoculated and the two who developed undoubted symptoms of plague within a few hours after inoculation. (Section 38): (*Dr Ruffer.*)—How many hours after inoculation?—The inoculations were performed between four and six o'clock (in the afternoon) and the buboes appeared the same evening<sup>1</sup>." (Section 44): "I repeat that the second conclusion drawn from the Byculla observations was that the prophylactic was powerless to arrest symptoms already started or which developed a few hours after inoculation. (Section 45): (*The President.*)—I understand that you mean in that dose?—Yes, certainly. The next deduction was that not only did the prophylactic do no harm to persons already infected, but that there was the possibility of its influencing the disease in the incubation period, in an individual infected three or four days previously. This conclusion was based on the following consideration. The examination of the occurrences which took place subsequent to the time of inoculation shows that, day after day, with the exception of one day, the fourth after inoculation, cases of plague continued to occur among the non-inoculated group of prisoners. The incubation period of plague, according to the facts collected up to now, appears to be between two and ten days. A large proportion of the non-inoculated patients were, therefore, likely to have been infected already on the day when we dealt with the prisoners. Such being the state of

<sup>1</sup> *Vide* the Commission's Report, vol. v, p. 197, table summarising the events in the Byculla House of Correction. The dates corresponding to what is referred to in that table as "Mr Haffkine's figures" are, as explained by me in a letter to the Commission, those of the first appearance in the patients of morbid symptoms; the dates corresponding to what is mentioned as the "Official figures" are those on which the patients were ultimately transferred from the observation ward to the plague hospital, and were reported officially as plague occurrences in the jail.

affairs in the non-inoculated group, and seeing that the inoculated had been living under the same conditions and had had the same chances of infection as the non-inoculated, I had ground to infer that a similar group had been infected in the inoculated also at the time when they were inoculated. Under these conditions, the reduction of the number of cases and the suppression of deaths among them pointed to the possibility of inoculation influencing the disease in the incubation stage." (Section 47): "The time necessary for the plague prophylactic to produce a useful effect is shorter than in any preventive treatment known, this period being in the anti-cholera inoculation four days, in vaccination against small-pox seven days, in the inoculation against anthrax 12 days, in the inoculation against rabies 15 days, and in the present treatment apparently less than 24 hours. This conclusion was drawn from the fact that the beneficial difference between the inoculated and the non-inoculated appeared from the next morning after inoculation. The third question which was to be decided was answered in this manner: the question was how long would the operation take to produce the required immunity? and the answer was, it required between 12 and 24 hours."

(Section 48): "In sending these observations to the authorities I added the following remark: 'The above conclusions are temporary and refer only to the teaching of the particular outbreak in question. There remains fully the possibility of further experiments compelling us to modify these conclusions, though the expectation is justifiable that the general bearing of the results as above detailed will remain unshaken.' The conclusions in question have, indeed, remained unshaken. In all the subsequent observations, the facts, collected under the strictest possible conditions, such as imitated the conditions of laboratory experiment to an extent probably not equalled in any other set of investigations, confirmed the deductions drawn from that first experiment."

The particular thesis here affirmed was demonstrated with great precision in a certain experiment at Undhera, in the Baroda territory, concerning which the Commission reprinted a detailed report of mine to Government. In an address which I delivered at the Royal Society in London, on the 8th of June, 1899 (*vide Proceedings of the Royal Society*, vol. LXV), and which the Commission made use of in discussing my evidence, I described the result of the operations in the village in question as follows:

"Deaths from plague among the non-inoculated and the inoculated

occurred after the following number of days had elapsed subsequent to the date of inoculation, viz.:

Among the non-inoculated:

after 3, 4, 5, 7, 8,—10, 11, 12, \_\_\_\_\_ 15, 16, 19, 20, 21, 24, 32 and 42 days;

and among the inoculated<sup>1</sup>:

after \_\_\_\_\_ 9, \_\_\_\_\_ 12 and 14 \_\_\_\_\_ days.

“There had elapsed, therefore, eight days, during which eleven deaths from plague had occurred among the uninoculated members of the families, before the first death took place in an inoculated case. The inoculation had again acted, so to say, immediately; or, as we have adopted to formulate the result, had acted within the time necessary for the subsidence of the general reactionary symptoms produced by the operation.” The Plague Commission, on its part, summed up the position during the first week of the epidemic in question in the following terms: “In Undhera, though plague continued among the uninoculated at the rate of ten cases in the first week after inoculation, there were among the inoculated two persons at most who can be regarded as having contracted plague at the time of inoculation.” (*Report of the Indian Plague Commission*, vol. v, 1901; p. 256, section 461.)

I further emphasized the thesis under consideration by separating (the Commission says, “excluding”) the events which had occurred within the first 24 hours after the date of inoculation, from the events which had occurred subsequently. In this way, in recapitulating the facts of the Byculla Jail, I stated that there had occurred in the morning of the day of inoculation, in non-inoculated persons, six plague cases, and in the evening, in inoculated persons, three cases, viz. one in a man who had a bubo at the time of inoculation, and two cases in whom buboes developed a few hours afterwards. Separately from these nine cases I cited the events of the rest of the period of the outbreak.

## B.

The following are the *Plague Commission's pronouncements with regard to the analysis of the Byculla Jail outbreak and the effect of inoculation in the incubation stage of plague* (vol. v, p. 197): “The second discrepancy relates to three patients who are excluded from consideration by Mr Haffkine in calculating his results on the ground that they had

<sup>1</sup> Half the members of each family had been inoculated.



already contracted<sup>1</sup> plague at the time when the inoculations were performed. If, however, cases are to be excluded for the reason that the full incubation period had not elapsed between the date of inoculation and the date of attack<sup>2</sup>, we should, assuming that the incubation period may last as long as five days, omit not only the three prisoners omitted by Mr Haffkine, but also every one who was attacked up to the 4th February<sup>3</sup>. If this be done, and we think that it ought to be done, there would remain seven cases among the uninoculated, with two deaths, contrasted with one case, which recovered, among the inoculated. The comparison would thus be greatly in favour of the inoculated. The results, however, do not seem to justify the conclusion drawn from them by Mr Haffkine that protection is acquired within 24 hours of inoculation, for during the first five days after the inoculation there were five cases among the inoculated as compared with four among the prisoners who remained uninoculated," a statement which was arrived at by placing the cases under the dates of admission to the plague hospital, instead of under the dates of the first onset of the disease in them (*vide* footnote on p. 67 above).

Page 255, section 461: "We have now to consider the possibility that certain of the less favourable results shown in the tables which are now under discussion may be due to the assignment to the inoculated of attacks and deaths due to infection contracted shortly before or shortly after inoculation....The experiment in the Byculla Jail is of particular interest in this connexion. If we compare the number of attacks that occurred among the uninoculated and the number among the inoculated from the date of inoculation, we find that there was a percentage of 6·9 and 3·9 cases respectively, among these two classes, giving a ratio of 1·8 to 1. But if, allowing five days for the incubation period, we exclude all cases that might have been incubating plague at the time of inoculation, we arrive at the results which have been tabulated above, and which give a ratio of 6·6 to 1." (P. 256, sections 462 and 463): "In view of the short incubation period of plague, and in view of the fact that our experience in the case of other diseases, both in animals and men, indicates that protection is not at all rapidly established, it seems to us unlikely that the anti-plague inoculation can

<sup>1</sup> Should be "developed."

<sup>2</sup> For summing up my reason (as quoted above) the last words should have been: "*had elapsed prior to the time of inoculation.*"

<sup>3</sup> In pursuance of the procedure adopted by me, only those who were attacked within 24 hours after the date of inoculation were to be "omitted." (See Section 47, quoted on p. 68.)



exert any favourable influence on persons who are already incubating plague. The matter, however, is one which can be definitely determined only by scrutinising the records of actual observation to ascertain how soon protection becomes established. We have, therefore, endeavoured to gather indications regarding this matter from the evidence laid before us.

“It would seem possible to test how soon protection is achieved by determining what percentage of the total attacks among the inoculated are attributable to each successive day or week after inoculation. We may consider first what details are necessary to apply this test. If we are dealing with a group of persons all inoculated on the same day, we should require to know the number of inoculated persons composing the group, the period for which they were kept under observation, and the number of cases that occurred among them in each day or week of the period of observation. It would, moreover, be necessary to know to what extent plague continued among the uninoculated during each day or week throughout the period of observation<sup>1</sup>, in order to determine whether the inoculated in the concluding days or weeks of that period were or were not exposed to less infection than those in the earlier days or weeks. We could then calculate out the daily or weekly percentage of attacks, for the first day or week, on the original number of inoculates, and afterwards, on the number of those remaining over unattacked on each day or week. A comparison of the percentages which occurred later would give the required information as to the date on which protection was achieved. When the results of a series of inoculations done on successive days in one community are in question, the same particulars regarding each successive group of inoculates will be required. That is not the method that has been adopted in the preparation of the statistics placed before us. The method adopted has been to bring the period of observation for a number of successive groups of inoculates to a conclusion on one and the same day, to sort out the cases that have occurred before that day into groups according to the length of time after inoculation at which they occurred, and then to compare the numbers of attacks in different days or weeks with a view to drawing conclusions with regard to the protection achieved at varying periods after inoculation. This method<sup>2</sup> leads to erroneous results. How erroneous these results may be will

<sup>1</sup> The operations in the Byculla House of Correction and in Undhera were performed at one sitting. The particulars enumerated by the Commission formed part of the description of the experiments in question.

<sup>2</sup> Not employed by me in any of my studies.

be manifest when we consider what would be the effect of applying the method to an extreme case. In such an extreme case as that of a community composed of two groups of inoculates, inoculated on two days separated by a considerable interval of time, if the period of observation was brought to a close within, let us say, a week after the second series of inoculations, it is plain that the ratios which the percentage of attacks occurring in the first week after inoculation would bear to the percentage of attacks occurring in weeks remoter from the dates of inoculation would be vitiated by the fact that the figures for the first week would include the cases that occurred in both groups of inoculates, while the figures for the subsequent weeks would exclude the attacks that might have occurred among the second group of inoculates after the date on which the period of observation was brought to a close."

## C.

The portion in *Prof. Wright and Drs Morgan, Colebrook and Dodgson's Report which bears on this subject* contains a table showing the results of inoculations with various doses of pneumonia bacilli. The table is as follows (*The Lancet*, January 10th, 1914, p. 91):

TABLE XVIII.

	Number in group	Number of cases of Pneumonia which developed													
		First day		Second day		Third day		Fourth day		Fifth day		Sixth day		First six days	
		Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
Group A (inoculated with 250 millions)	646	—	—	—	—	1	1	2	1	—	—	—	—	3	2
Control group	626	1	1	4	1	3	1	2	2	—	—	2	2	12	7
Group B (inoculated with 500 millions)	759	—	—	2	—	1	1	—	—	3	1	1	—	7	2
Control group	764	1	1	5	2	3	1	2	2	—	—	2	2	13	8
Group C (inoculated with 1,250 millions)	1,582	8	4	2	—	1	1	1	—	—	—	—	—	12	5
Control group	791	1	1	2	2	—	—	—	—	—	—	1	—	4	3
Group D (inoculated with 500 millions glucose vaccine)	463	1	1	—	—	1	—	—	—	—	—	—	—	2	1
Control group	457	1	1	3	1	3	1	1	1	—	—	2	2	10	6
Group E (inoculated with 1,000 millions glucose vaccine)	650	—	—	1	—	2	—	2	—	—	—	1	—	6	—
Control group	595	1	1	4	2	3	1	1	1	—	—	2	2	11	7
Group E (inoculated with 2,500 millions glucose vaccine)	1,582	7	3	11	2	1	1	3	—	2	2	—	—	24	8
Control group	791	1	1	2	2	—	—	—	—	—	—	1	—	4	3

The authors make the following comments on this table:

"The facts which are set forth in the table are, as will be seen, very remarkable. Associating together the figures which apply to Groups A, B, D, and E, *i.e.* the groups which received doses up to 1,000 millions of pneumococci, we find that, in the first four days after inoculation, 2,500 inoculated had an incidence-rate of 0.52 per cent., and a death-rate in connexion with these cases of 0.16 per cent., while 750 controls had an incidence-rate of 1.4 per cent., and a death-rate in connexion with these cases of 0.84 per cent. In other words, the uninoculated had an incident-rate nearly three times, and a death-rate five times greater than the inoculated.

"Again, associating together the figures which relate to Groups C and F—groups which received doses of over 1,000 millions of pneumococci—we find that 3,200 inoculated had for the same period an incidence-rate of 1.1 per cent., and a death-rate in connexion with these of 0.32 per cent., while 800 controls had an incidence-rate of 0.4 per cent., and a death-rate also of 0.4 per cent.

"Two important conclusions follow: the first is that pneumococcus inoculation undertaken with doses up to 1,000 millions had a marked effect in aborting pneumonia and in diminishing the case mortality. Or we may phrase it otherwise. Vaccine therapy as applied to the treatment of pneumonia is successful when doses of 250 to 1,000 millions are given in the incubation stage of the disease. The second conclusion is that inoculation undertaken with doses of over 1,000 millions of pneumococci may perhaps temporarily increase the incidence-rate of pneumonia.

"It is perhaps of interest to point out that these conclusions are essentially the same as those formulated in connexion with plague vaccine by Mr Haffkine, immediately after he had carried out his first mass-experiment in the Byculla Jail, Bombay, in 1898<sup>1</sup>. In that experiment, as in the mass-experiment we are here dealing with, a decisive difference in favour of the inoculated half of the population manifested itself already within 24 hours. And the view that Mr Haffkine maintained (in contravention to that held by one of us) that plague vaccine does not produce a negative phase, and that it has the power of aborting an incipient attack, was afterwards established by evidence accumulated by Miss Alice Corthorn, M.D., and Surgeon-General W. B. Bannermann, I.M.S. In connexion with this, all that requires to be said is that the generalisations in Section II of this

<sup>1</sup> In January, 1897.

Report—generalisations which have been reached only after years of further work—have made it intelligible that a negative phase should manifest itself with large doses of typhoid vaccine<sup>1</sup>, a vaccine which is easily broken down in the normal organism, and again with all vaccines after the organism has, by foregoing immunising response, acquired bacterioclastic power, and that this phase should make default in the uninfected organism, and in the early stages of infection when vaccines, such as plague vaccine and pneumococcus vaccine<sup>2</sup>, which are with difficulty broken down in the body, are inoculated."

## SECTION II.

In the quotation given in the preceding section Prof. Wright and Drs Morgan, Colebrook and Dodgson suggest, for the first time, I believe, that by the term "*vaccine therapy*" should be designated that form of vaccine treatment the effects of which were described in my evidence to the Plague Commission of 1898-99, viz. *the treatment by vaccine of patients in the incubation stage of the disease*. The notes which follow refer to *the extension of that treatment to patients in whom infection has progressed beyond the incubation stage* and is manifested by morbid symptoms.

### A and B.

On page 67 above are reproduced my statements to the Commission regarding the effect of inoculation when applied to a person

<sup>1</sup> *Vide supra* the statement as to the effect of large doses of pneumonia vaccine.—In carrying out the work of anti-typhoid inoculation, the operators introduced, amongst others, a certain formula for determining the volume of the dose, which the Plague Commission defined as "the quantity of broth culture which is lethal for 100 grammes of guinea-pig." (Vol. v of their Report, page 183, section 395.) My criticism of this procedure was made in a Report to Government, No. 1269 of 9th August 1900, "On the present condition of manufacture of the plague prophylactic in the Plague Research Laboratory," Government Central Press, Bombay, 1900; pages 14-17. It was seen shortly afterwards that the dose as above defined was apt to cause to the inoculated increased incidence of typhoid, or, in the terms of the statement quoted above, to produce "a negative phase." Subsequently, a special "Anti-Typhoid Committee," appointed at the War Office, and of which Sir Almroth E. Wright at first formed part, rectified the procedure as regarded the dose and some other particulars, and the inoculations, which had been suspended by the orders of the War Office, were resumed. (Cf. Sir W. B. Leishman, "Anti-Typhoid Inoculation," *Journ. of the Royal Inst. of Public Health*, July, August and September, 1910; and *Report of the Anti-Typhoid Committee*, 1912, His Majesty's Stationery Office, London.)

<sup>2</sup> Cf. the paragraph before the last and the second conclusion in the last.



very shortly before or very shortly after the appearance of plague symptoms, as compared with its effect when applied *prior* to the last stages of incubation; also the President, Sir T. Fraser's queries concerning the doses to which my statements referred, and my replies that they referred to doses effective when used prior to the final incubation stages.

The relative position of these two applications of "vaccine treatment" and my attitude on the question were indicated in a further query by the President and in my reply to it, which was as follows (vol. I of the Commission's *Report*, page 12, section 79): "(*The President.*)—Have you any definite reason to suppose that your substance is not purely therapeutic as well as preventive or prophylactic?—No, Sir. I have not subjected this question to any accurate examination; but the general impression which I have had up to now is that the inoculation is not likely to influence the course of the disease when symptoms have already started."

I described to the Commission what I considered an accurate examination of such a question (or, as I expressed myself on that occasion, what I thought to be "the only reliable method for finally testing a curative treatment"). I had subjected to such a test a serum prepared on the plan of Dr Yersin's curative serum for plague; and I stated concerning this as follows (vol. I, page 14, sections 140-141):

"We (my assistant and myself) visited the hospital daily, from the early morning, and took the name of every new patient admitted. With the exception of those who died within an hour or a few hours, that is before we could attend to them, we treated with plague-antitoxic serum every second patient admitted during the hours we were at the hospital, irrespective of the information as to the serious or promising condition of the patient, or the duration of the disease before admission. Without selecting patients according to our personal impressions or according to the statements supplied by the medical officer or by the patient or his relatives, we subjected to the treatment every second arrival. After about 200 patients had passed into the hospital, we compared the mortality statistics among the treated and among the non-treated. I expect it was an accident, but the mortality among the treated was higher than among the non-treated. The moment this became clear, we suspended further treatment....I consider this the only reliable method for finally testing a curative treatment. We suspended the treatment in Poona where we had injected considerable doses of the serum. Further attempts were made in Bombay with



some homoeopathic doses,—injecting 1 to 5 c.c. of serum, or so.... We varied the treatment in many ways. For instance, a patient would receive only 1 c.c.: or he would receive that amount repeatedly, every five hours; or again he would receive a dose of 10 c.c. once in two days. The patients were always observed comparatively with others admitted at the same time. In no case did we find a noticeable advantage on the side of the patients treated.”

As regarded the *prophylactic inoculation*, the beneficial effects obtained from it in the incubation stage, in the case of a disease of such rapid course as the plague, led me to admit the possibility of profitable results from it also in ailments actually developed, but having a long-standing, non-acute character, while some of my co-workers were induced to try the method even in the case of acute ailments. The queries of the President of the Plague Commission indicated that, on his part, he was inclined to believe in the possibility of good effects in the case of developed plague.

Soon after the Commission returned from India, and prior to the publication of their criticisms quoted on pages 69–72 above, viz. in October, 1900, Prof. Wright began to apply the principle therein under consideration, as a basis for therapeutic practice<sup>1</sup>, and since then, under the name of “vaccine-therapy,” the plan has been extensively used, in all infectious diseases.

### C.

In their present enquiries on pneumonia, Prof. Wright and Drs Morgan, Colebrook and Dodgson have, for the first time since its introduction<sup>2</sup>, submitted this form of vaccine treatment to the test described above in connexion with the studies of Yersin’s serum. They state the results as follows (*The Lancet*, 10th January, 1914, page 87):

“It will be well to realise at the outset under what disabilities of ignorance we here pursued our work. The methods of blood examination which so often disappointed us when we were endeavouring to compare from day to day the opsonic power of the inoculated with that of the uninoculated natives, left us quite in the lurch when we set ourselves to make similar daily measurements in the case of our

<sup>1</sup> *The Lancet*, 10th January, 1914, p. 92, quoted on p. 78 *infra*, and A. E. Wright, “Notes on the treatment of Furunculosis, Sycosis and Acne,” *The Lancet*, 29th March, 1902.

<sup>2</sup> *Vide* Part II of this memoir.

pneumonia patients. We were unable to trace upon 50 immunisation curves which we plotted out in connexion with this work the effect of the doses of vaccine which we administered.

"Accordingly, from first to last, we had to guide ourselves in our choice of doses and of the intervals between our doses only by *à priori* considerations, and by the uncertain and flickering light which is furnished by temperature charts and the clinical symptoms. Influenced by the anticipation that the infected natives would be much more sensitive to pneumococcus vaccine than the uninfected native, we employed only doses of  $2\frac{1}{2}$  to 50 millions of pneumococci; and we conformed to the principle of giving in the less serious conditions larger, and in the more serious ones smaller, doses. In the ordinary case we repeated the dose at intervals of 24 to 48 hours.

"These experiments—they have only the value of properly controlled reconnoitring experiments—were carried out in the hospital of the Witwatersrand Native Labour Association on tropical native patients. Many of these were, when admitted to hospital, already in an advanced stage of pneumonia. We accepted for our experiments only those who presented quite typical physical signs, and these were taken for treatment by vaccine-therapy or for treatment by the expectant method, alternately, and strictly in the order in which they were admitted to hospital. We took for every uninoculated patient who was treated by vaccine-therapy an uninoculated control, and for every inoculated patient an inoculated control.

"As the net effect of our treatment, we obtained the results which are set out in the subjoined table:

"TABLE XI.

*"Showing the Case-Mortality of Pneumonia in Tropical Natives treated respectively by repeated Small Doses of Pneumococcus Vaccine and by Expectant Methods.*

Therapeutic method employed	Number of cases	Number of deaths
Vaccine-therapy	159	50
Expectant treatment	149	48

"We would in connexion with these results specially emphasise (1) that they apply only to tropical natives who, having a very low power of resistance, have contracted virulent infection; and (2) that they apply only to inoculations carried out on such natives with the doses specified above."

Page 92: "In connexion with the vaccine-therapy of pneumonia we have, on the one hand, the fact that inoculation in the form of small doses frequently repeated was absolutely ineffective (Table XI); and, on the other hand, the fact that inoculation in the form of a single large dose, administered in the incubation period, often arrested the disease and averted death (Table XVIII<sup>1</sup>).

"That the difference of dose determined the difference of event, is to us as good as certain. Let us—recalling to mind the general propositions formulated in Section II—here take note of the fact that the doses which we found inoperative were doses from which there could, at best, have been expected that they should elicit a local immunising response. Further, let us note that the evocation of such response would be dependent upon a sufficiency of antigen passing into solution in the lymph at the seat of inoculation.

"Lastly, let us note that it is quite likely that microbes which are ingested by phagocytes may, from the point of view of the immunising reaction, be left quite out of regard. In connexion with this it is almost superfluous to point out that when comparatively small numbers of microbes are inoculated, and when they come into contact with a lymph which possesses opsonic power, but only inappreciable bacterioclastic power, they will almost certainly sooner or later be ingested by phagocytes.

"In general contrast with all this would be what would happen when a large dose of vaccine is inoculated. In this case the microbes would be carried on into the main lymphatic current or blood-stream, with the result that inevitably some would escape phagocytosis, and inevitably some of these would, even if the blood had but very little bacterioclastic power, be broken down. And there would supervene upon the convection of the antigen to the tissues through the blood a systemic immunising response.

"As we see no reason to suppose that the conditions appreciably alter, and as we know that the bacterioclastic power of the blood does not sensibly increase when pneumonia develops, we think it reasonable to expect that the favourable results which were obtained by the inoculation of doses of 250 to 1,000 millions of pneumococci would repeat themselves if this treatment were applied in the early stages of pneumonia."

<sup>1</sup> Reproduced on p. 72; *vide* also p. 75.

## SECTION III.

## A and B.

The present section concerns a subject which, though not clear to me at the time, I was obliged to refer to in a certain report to Government dealing with *the inoculations in the Khoja Community of Bombay*. The Indian Plague Commission, in discussing the report in question, constructed from a portion of its data a table of which it says (vol. v, pp. 209 and 210): "This table shows that among the inoculated deaths from plague were  $12\frac{1}{2}$  times less numerous, and deaths from general causes were 19 times less numerous, than among the uninoculated<sup>1</sup>. *Primâ facie*, therefore, it would appear that Mr Haffkine's anti-plague inoculation protects against plague, but that it protects more against ordinary diseases. This result is so striking and so difficult to accept that we first addressed ourselves to the task of enquiring whether the inoculated were a picked body, and whether the uninoculated contained a large proportion of the sick and feeble, and of the very young and very old. Surgeon-General Harvey, I.M.S., who made a special personal enquiry into the results of the inoculations performed in the Khoja Community, was of opinion that, in the main, the explanation of the disproportion between the deaths from general causes among inoculated and uninoculated Khojas must be sought in the assumption that in many families the sick, the weak, the elderly people, and the children, did not present themselves for inoculation, and that only the strong and healthy undergo the operation. In view of this opinion<sup>2</sup>, we have tested the proportion in which the different age groups were represented among the inoculated and the uninoculated Khojas respectively.... These percentages indicate that, in point of age at least, the two communities were not sensibly incomparable. In view of these percentages the next point to determine was whether the excessive mortality from general causes assigned to the uninoculated was really due to excessive deaths in any particular class of the uninoculated community.... From this table it will be apparent that excessive

<sup>1</sup> My own analysis pointed approximately to a proportion of 9.6 and 3.8, as against the Commission's  $12\frac{1}{2}$  and 19. Subsequent consideration has shown me that the figure relating to general causes (3.8) required to be further reduced, and that relating to plague (9.6), correspondingly increased.

<sup>2</sup> *Vide* foot-note (2) on p. 80.



mortality from general causes occurred in all three classes of the uninoculated community (*i.e.* in children under seven, persons of intermediate age, and old people of 61 and over). . . . In view of the facts that are thus summarised here, Mr Haffkine sums up the case of the Khojas in his Report as follows: 'After making all allowances for inaccurate classification of deaths in the uninoculated group, with which the inoculated is being compared, and admitting that a part of the excess of deaths in the uninoculated may be due to a certain number of sickly people having abstained from inoculation, the result still contains an indication that, besides the protection against plague, this inoculation influences also favourably the resistance to certain other diseases than plague.' We find ourselves in agreement with Mr Haffkine in holding that the difference in mortality among the inoculated and uninoculated cannot be fully accounted for, either by the excess mortality of the uninoculated children and old people, or by the incorrect assignment of plague deaths among the uninoculated to general causes. . . . Therefore, there remains to be considered, of the explanations offered by Mr Haffkine, only the suggestion that his anti-plague inoculation exercises a protective influence against diseases other than plague. This question is discussed elsewhere in the Report<sup>1</sup>. We cannot, however, accept Mr Haffkine's view that the low mortality among the inoculated can be accounted for on this hypothesis. It seems to us very probable, on consideration of all the circumstances, that the figures of mortality which have been given above must be accounted for by assuming that deaths which occurred amongst the inoculated were wrongly assigned to the uninoculated<sup>2</sup>."

<sup>1</sup> *Vide* next quotation.

<sup>2</sup> This view, and not the one quoted on p. 79, had originally been held by Surgeon-General Harvey, I.M.S. On 7th August, 1898, after first perusing my Khoja Inoculation Report, he wrote to me from Simla, in conjunction with the late Lieutenant-Colonel J. T. W. Leslie, I.M.S. (subsequently Sanitary Commissioner with the Government of India), as follows: "There must be a fallacy somewhere unless you have unconsciously hit upon the *Elixir vitae*. It seems to me that your figures make the uninoculated accountable not only for their own proper deaths, but for those among the difference between the total ultimately inoculated and the mean daily average of such." In the March following, upon my suggestion, he came to Bombay, made a detailed enquiry, which lasted several days, in the Khoja quarters of the town and ascertained that the view in question was not tenable. His statement to the Commission after this investigation was as follows ("Minutes of Evidence," vol. III, p. 347, section 26, 435): "I think that the records of the community are kept in a much more full and proper way than those of any average people. Several points have come out in addition to the fact that the records are correct. . . . We know, I should think, with practical accuracy from the Jamaat books those who



The Commission's ultimate statement on the subject was as follows (vol. v, p. 261, section 469): "Only one question now remains to be dealt with in connexion with the influence on the organism exerted by Mr Haffkine's anti-plague prophylactic fluid. This question relates to the suggestion, to which we have already referred, that anti-plague inoculation protects not only against plague but also against other diseases. This suggestion emanated from Mr Haffkine. It appears that in the course of his inoculation work a certain number of cases were brought to Mr Haffkine's notice in which fevers of an undetermined nature were favourably influenced by the injection of his vaccine. An idea that anti-plague inoculation might possibly protect against other diseases having thus suggested itself to him, Mr Haffkine proceeded to seek in this idea an explanation for the extraordinary absence of mortality from general causes which was noted in the case of the Khojas inoculated in Bombay. We have, however, pointed out that the exceptionally light mortality recorded for the inoculated Khojas is capable of being explained in quite a different way. What we have just said applies not only to the case of the Bombay Khojas, but also to the case of the inoculated Karachi Khojas, among whom there was a similar extraordinary absence of deaths from general causes, which was similarly attributed to the effects of inoculation. The instances which have just been referred to constitute the only statistical evidence which has been brought forward in support of Mr Haffkine's claim that his vaccine favourably influences diseases other than plague. As this statistical evidence is untrustworthy, we have to fall back on *à priori* considerations, the two isolated instances which Mr Haffkine has adduced of cases of fever favourably influenced by inoculation, and a few other isolated instances adduced by other witnesses. According to the more or less indefinite statements of the two or three witnesses who are in question, the plague prophylactic is capable of favourably modifying every possible class of disease, from ringworm to leprosy. It is obvious that statements of this kind are not deserving of serious attention. That vaccination against one disease may influence the

have been inoculated and those who have not, so that we can get a very fair index as to the mortality among the two classes." "I find that the original investigation of Prof. Haffkine was one which involved an enormous amount of energy and thought. Although it is a very short report (see App. No. iv in vol. i of these Proceedings), the amount of work involved in it was enormous."—Surgeon-General Harvey's statement concerning the Jamaat records referred, as he mentioned, to the assignment of deaths among the inoculated and the non-inoculated, and not to the diagnosis of the causes of deaths.

course of another is, however, *à priori* quite credible, inasmuch as it is known that one disease may influence the course of another. But since no trustworthy evidence has been adduced before us to show that this obtains in the case of anti-plague inoculation, the suggestion, though one that might be kept in view by future observers, need here no longer engage our attention."

## C.

The Report under consideration, by Sir Almroth E. Wright and Drs Morgan, Colebrook and Dodgson, does not refer to the Khoja investigations, but contains the following statements:

*The Lancet*, January 10, 1914, p. 91: "In concluding this account of the results obtained in mass-experiment No. 5 we may profitably advert to one more general consideration. It is, as will presently be brought out more fully in Section V, reasonable to expect that an effective inoculation will give an additional bonus in the form of a diminution in the morbidity which comes upon the record under the heading of 'Other Diseases.' In point of fact, the records which relate to the particular mass-experiment we are here discussing show such a reduction. We have our bonus in the form of a 15 per cent. reduction in the 'other diseases' of the inoculated, the figures being: *inoculated*, 6,224; *uninoculated*, 1,545. Cases of sickness other than pneumonia: in *inoculated*, 2,154; in *uninoculated*, 620.

"We now pass to our 6th and last mass-experiment."

Page 92: "In connexion with the mass-experiment here in question (the 6th and last mass-experiment) we may give the figures for the corresponding period relating to the incidence and death-rate of 'other diseases' in the inoculated and uninoculated sections of the population. These figures are as follows: *Inoculated*, average daily strength, 9,909; incidence-rate, 47.2 per cent.; death-rate, 0.93 per cent. *Uninoculated*, average daily strength, 4,520; incidence-rate, 106.6 per cent.; death-rate, 1.90 per cent."

In connexion with the same "mass-experiment No. 6" the authors give on p. 94 the following table:

"TABLE XXI.

*"Showing for the Whole Native Population of the Premier Mine the Incidence and Death-Rate for Pneumonia; the Incidence and Death-Rate for 'Other Diseases'; and also the Number of working Days Lost through Illness; for the Months February to May\*, in 1911, 1912, and 1913 respectively.*

	1911	1912	1913
Population (daily average strength)	10,426	12,549	15,284
Proportion of the population inoculated	0	About 50 per cent.	About 92 per cent.
Incidence-rate of pneumonia	4 per cent.	1.28 per cent.†	0.74 per cent.†
Death-rate from pneumonia	0.97 per cent.	0.31 per cent.	0.14 per cent.
Incidence-rate of other diseases	31 per cent.	20.7 per cent.	14.4 per cent.
Death-rate from other diseases	0.51 per cent.	0.38 per cent.	0.34 per cent.
Number of working days lost per hundred native labourers	275	177	131

\* "We have been furnished with data for this comparison only up to May, 1913."

† "In 1912 the incidence-rate was 0.86 per cent. for the inoculated and 1.7 per cent. for the uninoculated. In 1913 it was 0.6 per cent. for the inoculated and 3 per cent. for the controls."

## SECTION IV.

The subject referred to in the present section and in the one which follows concerns *the procedure for estimating numerically the effects of inoculation.*

## A and B.

In making out the *plague ratios for the inoculated and the non-inoculated* in certain epidemics, viz. in Lanauli and Kirkee, where the inoculations had been carried out, early in the outbreak, during a succession of days, I referred the incidence of the disease to the mean daily strength of the population. The Commission stated regarding this procedure that "very considerable complications are introduced when the incidence of plague has to be calculated on the average instead of upon the absolute strengths" (vol. v, p. 203); "the comparison which Mr Haffkine has made is unduly to the disadvantage of inoculation" (p. 205); "his calculation gives a slightly lower value for inoculation than that obtained by the calculation on absolute strengths. The result is as close an approximation as can be expected in applying the complicated method of calculating on averages to figures of an individual epidemic which are to a large extent the result of undetermined

causes" (p. 207). In drawing up a "Synoptical Table" of the results observed in various epidemics, the Commission, therefore, definitely put aside my figures based on average strengths, and recalculated the results upon ratios based on absolute strengths of population (vol. v, p. 251).

## C.

In their present publication, Prof. Wright and Drs Morgan, Colebrook and Dodgson give an analysis of "a detailed synopsis of results" obtained in a part of their operations in South Africa, and base all calculations from that synopsis on the "*Daily average Strength of the Group*," the "*Daily average population*," the "*average daily strength*" (Table XIX and text, p. 92 of *The Lancet*, January 10, 1914), or the "*Population (daily average strength)*" (Table XXI on p. 94).

## SECTION V.

## A.

Apart from the matter of ratios, the Commission differed from me as regarded the process which I had introduced for *calculating the protection obtainable from inoculation*. In studying epidemics from this point of view, I defined the question for enquiry thus: What percentage of plague cases and deaths had been averted by inoculation? The answer is supplied by the following formula, viz.:

$$100 \left( 1 - \frac{P_n \cdot C_i}{P_i \cdot C_n} \right),$$

where  $P_n$  and  $P_i$  represent the non-inoculated and inoculated portions of the same population, and  $C_n$  and  $C_i$ , the casualties observed in them.

## B.

The Commission expressed the view (vol. v, p. 252, section 454) that the above mode of enquiry was "likely to give rise to misunderstanding, especially when it is made, as it has been by Mr Haffkine, the basis for such a general statement as the following: 'One can say in a general manner that the reduction in mortality, produced by inoculation, is between 80 and 90 per cent.' Such a statement, taken apart from the actual figures, might be taken to imply either that inoculation had averted death from 80 to 90 persons (*a*) in every 100 of the total population, or (*b*) in every 100 of the inoculated population, or (*c*) in every 100 inoculated persons attacked, whereas the statement



really means that death was averted from 80 to 90 in every 100 of those who, without inoculation, would, judging by the mortality among the uninoculated, have died of plague." "With a view to avoiding such fallacies we have expressed our results in a form that is not open to these objections. In column 10 of our table we have set forth the ratio in which the deaths among the uninoculated stand to the deaths which actually occurred among equal numbers of the inoculated."

## C.

In the publication under review the authors have, throughout their analyses, resorted to the calculation and used the verbal expressions objected to by the Plague Commission, and have on no occasion stated the results in the form substituted by the Commission for mine. Thus on p. 91 of *The Lancet*, January 10, 1914, they refer, as already quoted, to "a 15 per cent. reduction in the 'other diseases' of the inoculated, the figures being: *inoculated*, 6,224; *uninoculated*, 1,545; cases of sickness other than pneumonia: in *inoculated*, 2,154; in *uninoculated*, 620."

The "15 per cent. reduction" here mentioned, or, more precisely, 13·76 per cent., is the result of calculation summed up in the above quoted formula, 1·16 being the figure which would have resulted from the procedure proposed by the Plague Commission.

On p. 93 of *The Lancet* the authors give the results of "mass-experiments" Nos. 1, 3, 4, 5 (E), and 6, as "a reduction of 37·5 per cent. in the death-rate of the inoculated"; "a reduction of 31 per cent. in the death-rate of the inoculated"; "a reduction of only 10 per cent. in the incidence-rate and of 34 per cent. in the death-rate"; "a reduction of 35 per cent. in the incidence and 55 per cent. in the deaths" (the latter figure being referred to shortly before as "a maximum reduction of 50 per cent. in the death-rate of the inoculated"); and "a reduction in the incidence-rate of 50 per cent., or 58 per cent., and a reduction in the death-rate of 52 per cent., or 61 per cent." (the latter figure being referred to previously as "a reduction of 60 per cent. in the death-rate for the inoculated").

These results have been calculated by the authors in the manner summed up in the formula quoted on the preceding page, the precise figures being respectively: 39·38; 28·09; 12·43; 34·32; 35·28; 54·23; 50·38; 58·03; 51·55 and 61·16 per cent. According to the procedure substituted for mine by the Plague Commission the results would have been stated in figures of 1·65; 1·39; 1·14; 1·52; 1·54; 2·18; 2·01; 2·38; 2·06 and 2·57 respectively.



## SECTION VI.

The foregoing notes refer to subjects on which the Indian Plague Commission of 1898-99 dissented from me, and concerning which Sir A. E. Wright and his co-workers, Drs Morgan, Colebrook and Dodgson, have now arrived at results coinciding with mine.

The Report under examination contains no findings of an opposite bearing; that is, among the matters that came within the authors' purview there were no subjects regarding which it appeared that the conclusions advanced by me required to be modified.

I propose, therefore, to complete this review by mentioning the same authors' finding on one further matter, which did not come under the consideration of the Indian Plague Commission of 1898-99, but on which other experts, called upon to advise on the plague, differed from me.

The matter concerns, indirectly, an opinion which I expressed in Poona, in January, 1898, to the effect that, independently from their mode of life, *certain human races*, like Europeans, Egyptians, Somalis, Kaffirs, perhaps also Arabs and the Felaheen, *appeared less receptive of plague than others*, the Chinese or Indians, for example; while in some respects, *e.g.* in regard to typhoid fever or the effects of the sun, the mutual position of Europeans and Indians appeared the reverse of the above. In an address delivered by me, in December, 1907, before the Royal Society of Medicine, in London, and entitled "On the present methods of combating the bubonic plague<sup>1</sup>," the subject of racial differences in regard to plague was referred to in five of the six following propositions mentioned by me on that occasion, viz.:

"(1) That in a native of that country (India), who is more susceptible to the disease than Africans, Europeans and some other races, the inoculation now in force in India reduces the liability to attack to less than one-third of what it is in a non-inoculated Indian.

"(2) That in the one-third of cases which still occur, the recovery rate is at least double that in the non-inoculated attacked, the ultimate result being a reduction of the plague mortality by some 85 per cent. of what it is in non-inoculated Indians.

"(3) That in an inoculated European an attack of plague, if it subsequently occurs, has so far always ended in recovery.

<sup>1</sup> *Proceedings of the Royal Soc. of Med.*, January, 1908.

“(4) That the inoculation is applicable to persons already infected and incubating the plague, and prevents the appearance of symptoms, or else mitigates the attack, a fact which disclosed a basis for bacterio-therapeutic treatment of diseases.

“(5) That in natives of India the degree of immunity conferred by this inoculation, though gradually vanishing, seems to last during several outbreaks of plague; and that

“(6) In Europeans the effect has not yet been seen to disappear in the space of time, since 1897, that this inoculation has been under study.”

The publication by Sir A. E. Wright and Drs Morgan, Colebrook and Dodgson contains, on p. 10 of *The Lancet*, January 3, 1914, statements as to differences observed in Europeans and Africans in regard to the effect of germs on their respective bloods, the authors' conclusion being that the Africans have an advantage over Europeans as regards natural resistance to microbes of abscesses and suppuration, while Europeans have an advantage over the Africans as regards similar resistance to pneumonia. On p. 95 of *The Lancet*, January 10, 1914, the authors sum up their finding as to the germ of the latter disease by saying that “the blood of the African native is, so far as relates to its power of phagocytosing and killing the pneumococcus, very inferior to that of the European, and that the capacity for immunising response is also much less in the African than in the European.”

In connexion with the above remarks, I would mention that, soon after the present epidemic had spread over the plains of India, and shortly before the Commission of 1898-99 began their work, viz. in June, 1898, I endeavoured to obtain a modification of the general measures devised against the plague, in favour of persons who had undergone preventive inoculation.

The modification in question was indicated by the analysis of the main features of the outbreak, which showed that, for vast masses of the population exposed to the disease, *personal immunisation was the only accessible means of protection*.

I recognised at the time that the policy advocated would be unavoidably retarded by the existing divergence of views, and would be adopted only after a prolonged expenditure of effort in many other directions.

These conclusions regarding the measures for protecting the population in the plague epidemic areas are now shared, I believe, by many authorities; and the publication with which the present Note is concerned is some sign that unanimity is attainable also on questions regarding the nature of the prophylactic inoculation. Further progress is, however, required in many directions.

## PART II.

### *On experimental Study of curative Methods.*

#### I.

The description quoted on p. 75 *supra* of the mode of testing the *Yersin plague serum* in the Poona Hospital was given by me to the Indian Plague Commission on the 30th November, 1898, and was preceded by the statement which is reproduced below (*vide* "Indian Plague Commission, 1898-99. Minutes of Evidence," vol. I, p. 14, section 140):

"At first, while I had only a small quantity of serum at my disposal, I attempted to see its curative effect in the following way. I visited the Arthur Road Hospital, which had the largest number of plague patients in Bombay, and requested the Medical Officer in charge to point out to me those cases which he considered *hopeless*; that is, cases which, he thought, could not recover without the assistance of some new, additional treatment. He told me that he had a patient who had had, on the day before, a temperature of 107°, and that up to that time not one patient had passed through the hospital who had recovered after having had that temperature. In the same manner he pointed out to me a certain number of other patients who, in his opinion, had no chance of recovery. I reasoned that, if a proportion of such patients recovered under the specific serum treatment, we would have an indication that the treatment under enquiry produced a greater effect than the ordinary treatment adopted in the hospital. Very soon I had to abandon that plan, as no such indication was obtained. A curative treatment which has not the power of helping in critical cases may, however, still be useful. When the hospital authority sees before him, say, a hundred patients, there are, of course, amongst them three categories: one which will for certain recover,

another which will for certain succumb, and a third category in whose case the balance, by a useful treatment, may be turned towards recovery. The serum, even when powerless with regard to the second category, would prove valuable if it were to assist with regard to the third group of patients. I suspended temporarily further attempts until I had accumulated a sufficiently large amount of serum; and then went to Poona where the plague epidemic was on the increase, and the number of patients admitted to the General Plague Hospital was between 20 and 30 per day."

## II.

At the time of giving the above explanations to the Commission I dealt with the recovery rate, which, in an acute disease like the plague, clearly dominates all other questions. Subsequently, I applied the same plan of study to all *clinical features of that disease*, or, more precisely, to all those features which were reliably observed and recorded. This included symptoms and particulars which were common to all categories of plague patients, as well as those which were observed only in the distinctive types of the disease. In this manner the effect of the treatment was investigated in relation to the age, sex, nationality and caste of the patients; their condition as to previous inoculation; the duration of illness prior to admission to hospital and prior to the application of the specific treatment; the state of consciousness or coma on admission; the type of the illness, viz. the presence or absence of outward lesions (buboes); the number and mode of distribution of these, if present; the condition as to pneumonic symptoms; the detection or otherwise of germs in the circulation at stated periods of the disease; the temperature, pulse and respiration at the time of arrival at hospital and of commencement of treatment; the variation of these particulars after the first administration of serum and throughout the time of observation; the duration of febrile symptoms in recovery cases; the recurrences, if any, of high temperature and high rates of pulse and breathing subsequent to the first restoration of normal conditions; the recovery rate corresponding to variations of treatment; and the prolongation, if any, of life in fatal cases. The relative gravity of each of the above symptoms was at the same time minutely investigated. Of the observations which, at that period, were taken and recorded with regard to plague patients, I omitted, for the time being, to take account only of those which conveyed general opinions of the



observers, or were based on mental records, that is, those in the estimation of which personal inclination and idiosyncrasies, not admitting of control and verification, played an important part. In no case, however, were general impressions of this nature required for forming definite conclusions.

The studies here referred to were made with the serum of Prof. Lustig of Florence, which was used in Bombay in 1899-1900; with Drs Terni and Bandi's serum, from Messina, used in 1903 and 1904; and with Dr Vital Brazil's serum, from San Paolo, Brazil, and Drs Roux and Yersin's serum, from the Pasteur Institute in Paris, which were tried in 1904. Detailed reports, in each case, were submitted to Government, and a budget of these was subsequently edited and published, during my absence from India, in the *Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India*, No. 20, under the title of "Serum-Therapy of Plague in India, reports by W. M. Haffkine and Officers of the Plague Research Laboratory, Bombay." (Office of the Superintendent of Government Printing, India, Calcutta, 1905.) Subsequently, I made analogous experiments with the sera prepared by Dr Macfadyen and Prof. R. T. Hewlett for the treatment of plague and of cholera. A few of the facts established in these investigations will be briefly recalled here with the object of indicating the nature of the information thus obtained. The result showed that the injections tended to mitigate the symptoms of the disease, to an extent which was carefully determined with regard to each symptom. At the same time the fact first communicated to the Plague Commission was observed again, viz. that the remedy had no appreciable effect on the mortality from plague.

In the case of *Dr Lustig's serum*, the patients, 484 in number, presented the most marked difference in the rate of mortality as compared with the 484 patients not treated with serum. The proportion was 68.18 per cent. of deaths amongst the serum patients, and 79.55 per cent. amongst the others. Examination showed, however, that the patients who had received serum included a clearly defined preponderance of cases which had, from the first, comparatively benign symptoms, whilst those left without serum had a preponderance of grave cases. The average survival in hospital of the patients treated with serum, but who ultimately succumbed, was 3.89 days, while that of the non-serum patients was 2.76 days. Among the patients admitted with fever and given at once a serum injection, the proportion of those in whom the temperature fell within 24 hours and did not



again rise to the level of the admission temperature, was 27·95 per cent. Among the patients admitted in a similar condition and not treated with serum, the proportion was 23·83 per cent. The fever, however, did not disappear in the serum treated patients sooner than in the non-serum patients. Indeed, the number of cases in whom the maximum temperature was over within the first 24 hours of admission formed 68·14 per cent. of non-serum patients and 64·20 per cent. of serum patients. In the most serious of the non-serum cases, who recovered, the temperature became permanently normal in an average of 19·95 days; in the corresponding serum cases, in an average of 21·05 days.

Among the patients treated with *Terni and Bandi's serum* the mortality was, in one hospital, 89 deaths in 110 cases, or 80·90 per cent., while among the non-serum cases, admitted to the same hospital at the same time and alternately with the above, it was 90 deaths in 110 cases, or 81·81 per cent. In another hospital, the figures were 12 deaths in 16 serum cases, or 75 per cent., and 11 deaths in 16 alternate non-serum cases, or 68·75 per cent. When the two hospitals are considered together, the mortality is 80·16 per cent. in either category of patients. Taking the case of the hospital with the larger number of patients, the serum treated patients, who ultimately succumbed, lived, on the average, eight hours longer, after admission to hospital, than the others; the figures being 3·27 days and 2·93 days respectively. Advantage from an early administration of serum was not consistently observed; nor did cases in less susceptible ages, or with a less dangerous manifestation of buboes, or with a lower admission temperature, benefit by injection more than severer cases. On the other hand, patients admitted with a less dangerous condition of the circulation or breathing appeared to be so benefited. The proportion of patients who were treated with serum, and who, within 24 hours, showed improvement of condition, was, with regard to temperature, 58·22 per cent.; with regard to circulation, 46·42 per cent.; and with regard to breathing, 36·47 per cent. Among the non-serum cases the corresponding figures were 45·78 per cent., 32·55 per cent. and 34·88 per cent. Similarly, the proportion of cases in whom the gravest condition of temperature, circulation and breathing was passed in less than 24 hours after admission, was, for the serum cases, 38·09 per cent., 20·31 per cent., and 17·46 per cent., and for the non-serum cases, 26·31 per cent., 13·55 per cent. and 10·00 per cent. respectively. Yet, ultimately, the mortality in all these groups of cases was higher in the serum patients

than in the others. In the patients treated with serum, who ultimately recovered, the highest temperature and rate of pulse and respiration showed an average of  $102.6^{\circ}$ , 127 and 37 respectively; in the corresponding non-serum patients the figures were  $102.8^{\circ}$ , 138 and 41. Among the serum treated cases there were also fewer relapses of high temperature than among the non-serum cases. The recovery rate was not improved by increasing the initial dose or the total amount of serum.

In the case of *Dr Brazil's serum*, the mortality was, in one hospital, deaths in 20 serum cases (85 per cent.), against 15 deaths in 20 non-serum cases (75 per cent.); and in another, 41 deaths in 50 serum cases (82 per cent.), against 45 deaths in 50 non-serum cases (90 per cent.). When the two hospitals are considered together, the rates are 82.86 per cent. for the serum patients against 85.71 per cent. for the others. The difference in the two hospitals was again traceable to the inclusion of a few more serious cases in one of the two groups of patients. In both hospitals the injections tended to reduce the temperature and pulse rate within the first few hours of admission, the degree of that tendency being manifested in figures analogous to those mentioned above for the other sera. In both hospitals the serum patients who ultimately succumbed lived longer than the non-serum cases, the average advantage being, in one hospital,  $7\frac{1}{2}$  hours, and in the other, 2.55 days. The hospital which showed a longer survival of the serum cases had also a further advantage over the other in that the recovering patients, in the serum group, reached normal temperature, pulse and respiration sooner than the non-serum recovering patients; yet the ultimate mortality of the serum cases in this hospital was higher than in the other hospital, and higher than among its own non-serum cases, thus showing again the absence of a parallel between the improvement of symptoms and of the ultimate result.

In the case of the *Roux-Yersin serum*, the mortality was 45 deaths in 68 cases, or 66.17 per cent., among the serum treated cases, and 41 deaths in 68 cases, or 60.29 per cent., among the others, the difference being referable in this case also to the condition of the respective patients at the time of admission. Notwithstanding the unfavourable termination of the disease, the serum cases, who ultimately succumbed, lived, in this as in all the other instances, longer, after the date of admission to hospital, than the non-serum fatal cases, viz. 7.57 days as compared with 4.19. The proportion of cases whose highest temperature and pulse-rate became lower within the first few hours of arrival

in hospital, was 42·64 per cent. and 42·64 per cent. respectively in the serum group, and 39·85 per cent. and 32·35 per cent. in the other; yet the mortality, in each of these categories of serum cases, was higher than in the corresponding categories of non-serum cases. As regarded respiration, the figures were in a reversed order, viz. 38·23 per cent. for the serum cases and 41·17 per cent. for the others; and the mortality was in favour of the serum patients. In the case of this serum, as in the case of that made by Lustig, the maximum temperature and rate of pulsation and breathing were passed in the serum cases later than in the others; but, ultimately, the serum cases, which recovered, reached the normal, in respect of all these particulars, sooner than the others, the average delay being, for the serum cases, 6·24, 8·40 and 7·04 days, and for the others, 8·42, 9·20 and 9·02 days respectively.

### III.

Sir Almroth E. Wright and Drs Morgan, Colebrook and Dodgson published their Report referred to in the preceding pages in two instalments. The results of the application of "vaccine-therapy" to pneumonic patients (*vide* pp. 77-78 *supra*) were given in the second instalment, which was issued in January, 1914. In December, 1912, the authors published the first part of their Report, and therein they stated their views on *the method of investigation* by which those results had been established. The method was that dealt with in the foregoing Section, and which, as stated above, had been applied by me, in 1898, to the examination of the Yersin treatment for plague, and in 1899 to 1904, to a study of Lustig's, Terni and Bandi's, Brazil's and Roux and Yersin's sera. Prof. Wright and his collaborators refer to that method under the name of "the statistical method," adverting by this to the fact that experimental study proceeds throughout, and of necessity, by careful measurement and calculation<sup>1</sup>. They contrast this method with "*the experiential method*," by which name they designate the process of taking into account the complex of impressions which have been left on the minds of practising medical men in the course of their experiences.

The authors say (*The Lancet*, December 14th, 1912, p. 1636):

"If we employ the *experiential method*—i.e. if we take into account the whole complex of impressions which have been left upon the mind

<sup>1</sup> *Vide*, however, on p. 98 *infra* the authors' reference to the "popular verdict on statistics."



by experience, we arrive at a *generalisation* (which is the general law or general evaluation of the class). This will express the result which we have witnessed in the majority of our cases; and it will, if our experience has been a typical one, hold good of a majority of every other series of such cases. If, on the contrary, we proceed by the *statistical method*—i.e. if we tabulate and count up our results—we arrive at a *statistical evaluation*. This will set forth the percentage of cases in which a particular result was achieved; and it will, if our experience is a typical one, give the correct odds in favour of that result reproducing itself in the case to which we are giving our attention.

“An objection will already have suggested itself: ‘Is it,’ it will be asked, ‘beyond question that what is here called the “experiential method” is properly distinguishable from the statistical method? And does not the distinction between the methods consist only in this, that when we bring into application the so-called “experiential method” we are relying upon a badly kept and blurred mental record of the facts which warrants at best an evaluation in general terms, while we have in connexion with the statistical method an accurately kept written record which warrants an evaluation in precise figures?’ The question may be answered by analysing somewhat more minutely first the statistical and then the experiential method.

“The statistical method of evaluation involves three separate operations: (1) a critical study of the raw material of experience with a view to selecting a suitable criterion upon which to build up our statistics; (2) the sifting of that raw material by aid of the criterion we have chosen<sup>1</sup>; and (3) the evaluation of the results which the sifting has yielded.” (P. 1637): “The experiential is a much less sophisticated and a much less arbitrary method of evaluation. When we employ it we let the two separate streams of experience which correspond to the twin series of substantive and control experiments filter through our minds, and then compare the impressions which have been imprinted. While no complete account can be given of the psychological processes by which this comparison is carried out, the two following points may be noted. When we bring into application

<sup>1</sup> As may be seen from the brief summaries given on pp. 88–93, the plan of study described here as “the statistical method” embraces the *whole* of the “raw material of experience,” the selection of one feature as a criterion, to the exclusion of others, not being necessary. In acute diseases, however, often one particular “feature,” such as the recovery rate, may, indeed, be taken as an essential criterion of success. As already stated, I limited my enquiries to such a criterion in the first days of the plague epidemic in Bombay.

the experiential method we take into consideration every feature of each case and not, as in the case of the statistical method, only one selected feature<sup>1</sup>—in other words, the *experiential* is a method of *unrestricted*, the *statistical* a method of *restricted outlook*. And the mental record upon which we proceed need not be inaccurately kept or blurred. When, for instance, we obtain in a succession of consecutive cases one and the same result, each succeeding case will render more distinct the impression made by the preceding case; and when a case which is at variance with a series of previous cases turns up, it will by contrast stand out very clearly in our consciousness.”

Having defined the two methods, the authors state, regarding *the application of the “experiential method”*: “The capacities of the human intellect are unequal to the task of carrying in mind and weighing one against the other a long procession of substantive and a long procession of control cases. There attaches to the use of the experiential method also another important limitation. It is impracticable, in the case where the evaluation of different observers diverge, to bring these together into a single judgment. For there is no method for finding the resultant of a number of non-numerical evaluations.” Against this they point out, as *the disadvantages of the “statistical method,”* “that it is the exception to find in connexion with clinical material either a really critical feature by reference to which the cases can be sorted out into successes and failures, or a significant feature which is universally present and which lends itself to arithmetical evaluation<sup>2</sup>.” “As a certain counter-weight against the disadvantages we have been considering is to be reckoned the fact that, once a satisfactory basis for statistics has been found, we can obtain in the form of a single expression the resultant of the evaluations of any number of independent observers.” (*The Lancet*, December 21st, 1912, p. 1701): “Where such a criterion is not available, and where the personal judgment of the observer is called into requisition, it is very difficult, for him, in going through a long series of cases, to maintain exactly the same standard of value. The observer’s estimate of what amounts to an “attack” or a “relapse” or a “cure” will, for instance, vary; and it will be impossible in the case of different observers to obtain conformity to a uniform standard<sup>3</sup>.”

<sup>1</sup> *Vide* preceding foot-note.

<sup>2</sup> *Vide* foot-note (1) on p. 93 and remarks on pp. 88 and 90 concerning symptoms and particulars not universally present.

<sup>3</sup> *Vide* remarks on p. 90 on the idiosyncrasies of observers and on the question of recurrences of morbid symptoms.



Lastly, a comparison of the methods is made in the following respect (p. 1637): "When we evaluate by a method of restricted outlook, which takes into account only a single feature in each case (and we have seen that the statistical is such a method<sup>1</sup>), we must, before a trustworthy conclusion can be arrived at, pass in review a very large number of cases. When we evaluate by a method of unrestricted outlook (such as the experiential method) in which every feature in each case is taken into account, a much smaller number of cases will suffice. When there is a shorter and a longer way it will be well not to choose, *on principle*, the longer."

In the passages which now follow the authors refer to the value of the "statistical method," or "numerical evaluations," in medical research. They state (p. 1701):

"The suggestion which is here put forward is not that all numerical evaluations should disappear from medical literature. We shall have conceded to them their proper relative rank in science when we have put them upon a level with evaluations expressed in round numbers, and with approximate evaluations such as are obtained by the experiential method. They must, however, from the standpoint of ethics, rank below these. For, while approximative experiential evaluations are neither more nor less than what they purport to be, a precise numerical evaluation is a concession to that human weakness which insists that it must always be allowed to achieve, even at some sacrifice of truth, an absolutely definite mental image." "If we desire to learn in connexion with each individual only whether he remains well, or falls seriously ill, and whether he dies of his illness, or survives, the required statistics can quite well be compiled by perfectly unskilled labour. And it is by such agency that statistics are commonly compiled. But if we desire to learn what is the number of men who really fall ill of pneumonia, and the number who really die from it, the clinical skill that will have to be requisitioned will not be less than for an experiential evaluation." (P. 1702): "It has, in the course of the preceding analysis of the experiential and the statistical methods, been elicited that the statistical method employs, for purposes of numerical notation, a quite arbitrary scale of values; that it is, in contrast to the experiential method, a method of restricted outlook; that there are a very large number of cases to which the method cannot be applied; that it is not capable of bringing to light finer differences<sup>2</sup>; and that it demands,

<sup>1</sup> *Vide* foot-note (1) on p. 93.

<sup>2</sup> *Vide*, with reference to the four points mentioned, Section II, pp. 88-93 *supra*.

if the results are not to be of inferior value to those of the experiential method, the application of exactly the same measure of clinical skill."

"*Do considerations of intellectual morality prescribe that the statistical method should everywhere be brought into application?*" "The statistical method is believed to provide effective safeguards against moral shortcomings on the part of the observer and evaluator." "The observer who is blinded by intellectual, personal, or financial bias, but is constitutionally honest—and this is the type of observer against whom we have to be upon our guard—need only employ the statistical method to find himself estopped from overrating the cases which bear out his theories; and from underrating or putting out of sight cases which have turned out inconveniently for those theories. The experiential method gives opportunities for such departures from morality." "But there is something more to be taken into consideration. (a) It is important in connexion with the question of the warping of the observer's judgment by bias to realise that this cannot really count as a very formidable obstacle to scientific advance." "Bias, begotten as it is of self-interest, will affect only the verdicts of the original observer and of those who have definitely taken sides for or against him, and the rest of the world will be unprejudiced." "Whenever the statistician has free choice in the matter of the criterion which is to govern his classification—and he very often has such free choice—he can turn this choice to advantage in the interest of the particular cause which he happens to have at heart<sup>1</sup>." (b) "When we come seriously to make enquiry whether in the case of statistics the cards are really on the table, the answer must inevitably come that the mere setting down of the serial numbers, or, as the case may be, of the names of the patients, is not a fulfilment of the ideal of setting forth the data in such a manner as to make it possible for the reader to control the judgments of the observer." (c) "While it may be permissible to rank all men as equally competent observers with respect to things that admit of being measured by carpenter's rules, or of being weighed upon grocer's balances; and to rank as the most authoritative on these matters, the man who reports the largest number of observations; this doctrine cannot find application in medicine; for here in many cases truth can be arrived at only by exceptional skill and a very delicate calculation of probabilities." (P. 1703): "Looking back now over what has preceded, it will be borne in upon the reader, on the one hand, that medical statistics are nothing more than the data of imperfect clinical methods set out in

<sup>1</sup> *Vide* foot-note (1) on p. 94.

unwarrantably precise figures; and, on the other hand, that we have in connexion with the statistical method, just as with the experiential method, to reckon with opportunities for the intrusion of bias; with a defective realisation of the ideal of a complete disclosure of the data; and with the assumption of unwarranted authority on the part of the evaluator. The reader will probably have arrived at the conclusion that there is in all these respects little or nothing to choose between the two methods of evaluation<sup>1</sup>."

In the concluding passages, after criticising Prof. Karl Pearson's views on the value of expert opinion, when not supported by accurate data, the authors refer to the following "objection in the minds of the unthoughtful." They state (p. 1704): "The train of reasoning which commends itself to these runs somewhat as follows: 'Unanimity of expert opinion does not furnish any real guarantee of truth. If it did, the medical profession would not, time and again, have accepted unanimously—as it did for instance in connexion with blood-letting—experiential conclusions which the progress of knowledge has compelled it to abandon. And now just let me ask,' concludes our objector, 'is it conceivable that the medical profession would not have been saved from such gross errors if it had brought into application the statistical method?'" Concerning this the authors state that "it was not the bringing into application of the statistical method, but the undertaking of control experiments—that is to say, the treatment of patients without bleeding, and the comparison of these by the experiential method with the previous cases treated by bleeding—which led to the general abandonment of blood-letting<sup>2</sup>"; and that if a method of evaluation was to be discredited by the fact that an erroneous conclusion has been arrived at by its means, "both the experiential and the statistical methods of evaluation, but *in primis* the latter—upon which the popular verdict is that 'statistics will prove anything'—would be irretrievably discredited." "The doctrine of the probative value of a consensus of expert opinion is in no way invalidated by such a train of reasoning as that which we have been reviewing. In reality we are all of us recording machines—recording machines of the most diverse patterns—and when each several machine registers one and the same impression the correctness of such record is established beyond doubt. The

<sup>1</sup> *Vide* Section IV, p. 99.

<sup>2</sup> *Vide* the view quoted on p. 100 concerning the capacities of the human intellect for carrying in mind and weighing one against the other processions of substantive and control cases.



general sense of mankind proclaims this in the dictum: *Securus judicat orbis terrarum*. It does so again in the formula: *Quod semper; quod ubique; quod ab omnibus*. And if it stands fast that what is given in the experience of all is true, how shall this not hold also—also with the proviso that untreated as well as treated cases are included in every experience—in our difficult and distracted science of medicine?"

## IV.

In the interval between 1900, when Sir Almroth E. Wright first began to use "vaccine therapy" for purposes of professional practice, and the date of the South African experiment, he and his co-workers at St Mary's Hospital were, on one occasion, prevailed upon to try and ascertain the efficacy of that procedure in the way which had been commended by me, for such purposes, to the Plague Commission of 1898-99 (pp. 74 and 76 *supra*), and in which I had been testing curative treatments in India. The experiment on that occasion was not completed by them, and although the preliminary information which they published was favourable to the therapy, they did not persevere with the study, and the further use of the "statistical" or, as it was termed, "quasi-statistical," method was discouraged by them<sup>1</sup>. In the Hospital of the Witwatersrand Native Labour Association, in South Africa, the experimental testing of "vaccine therapy," by that method, was, therefore, carried to a conclusion for the first time since the therapy had been used in practice. The disease—pneumonia—on which the trial was made, was that in which, of all the graver infections, "vaccine therapy" promised to be the most successful<sup>2</sup>. The details of the actual experiment tabulated herein on p. 77 *supra* show that, of a total of 159 patients who were treated with "vaccine," 109, or 68·55 per cent., recovered; 149 others, admitted to the Hospital during the same time and alternately with the above patients, were, in accordance with the Poona procedure, treated by the current expectant method, and of these patients 101, or 67·78 per cent., recovered. The experiment having thus shown that the application of "vaccine therapy" made little difference, Prof. Wright and the other workers at the Hospital recorded

<sup>1</sup> *Vide* J. Freeman, M.D., Assistant in the Department of Therapeutic Inoculation at St Mary's Hospital, in the *Proceedings of the Royal Society of Medicine*, 1910, vol. III; or in J. Nachbar's "Vaccine therapy: its Administration, Value and Limitations," 1910 (Longmans, Green and Co.), pp. 99 and 100.

<sup>2</sup> *Vide* Sir A. E. Wright's address in the *Proceedings of the Royal Society of Medicine*, 1910; or in Nachbar's work just quoted, pp. 13 and 32.

that the treatment "was absolutely ineffective," and they recognised that the verdict, in respect of the treatment tried, was final.

On the subject as to whether information of that definite character was obtainable by the "*experiential method*" the following statements by the authors, partly quoted already, contain, no doubt, the reply (*The Lancet*, Dec. 14, 1912, p. 1637): "The experiential method will be inapplicable in the case where the substantive and control cases are distinguished by only a very small average difference." "The capacities of the human intellect are unequal to the task of carrying in mind and weighing one against the other a long procession of substantive and a long procession of control cases." (*The Lancet*, Dec. 21, 1912, p. 1701): "We have already seen that our inability to carry in mind and evaluate such long sequences of cases as are, in the case here in question, indispensable, makes it futile to employ the experiential method for the detection of fine differences," that is, as in the above instance of pneumonia, for the detection of ineffective lines of treatment. It is also the case that the "experiential method" allows of the complementary misapprehension, that is, of the results of treatments, in reality ineffective, being believed for a long time to be indications of success.

These facts notwithstanding, the application of the other mode of study, "the statistical method," by which the Witwatersrand information was successfully obtained, is not countenanced by the authors under review, as has been observed already; and the grounds leading to their opinion, as well as the purport of the general analysis of methods, by which the publication of the Witwatersrand result has been prefaced by them, are stated as follows (p. 1701): "These general considerations have prepared the way for bringing forward the suggestion that the ideal of minutely accurate quantitative statement which is always floating before the vision of the statistician should in the field of clinical medicine be frankly abandoned. This would mean recognising that it is, in medicine, impossible by the method of cumulative experiments either (*a*) to detect minute differences, or (*b*) to arrive in any case at any accurate quantitative conclusions<sup>1</sup>. The frank recognition of this would, in point of fact, leave the practice of medicine practically unaffected. For, both in the case where the question arises whether we are, or are not, to apply a method of treatment which is doubtfully effective, and in the case where we have to elect between two alternative lines of treatment which are almost equally effective, it will not seriously matter what choice we make."

<sup>1</sup> Cf. Section II, pp. 88-93 *supra* and the results of the Witwatersrand evaluation.



The latter conclusion will probably meet with some dissentient opinion, as the choice is apt to matter from the patient's and also from the physician's points of view. In the "vaccine therapy" treatment with which the authors were concerned in the Report referred to, the plans devised notoriously involve complicated procedures for the operators with a correspondingly great amount of bodily pain and trouble, and material sacrifices for the patient. There is sample-taking and microscopic and other scrutiny of the patient's lesions and blood; "autogenous vaccine," simple or "polyvalent," is manufactured in a laboratory for the individual use of the patient under treatment, if circumstances permit, and repeated appointments are required for injections of that "vaccine" and for one or several successive sample-takings of blood after each injection; technical work is again gone through in a laboratory, subsequent to each sample-taking, for the determination of the "opsonic index" and the plotting out of an "immunisation curve," and for the selection of the time and dose for the next injection, and so on. The knowledge that a complex plan of treatment like the one outlined, or a treatment on similar lines, does not exceed in efficacy an "expectant method," and is, in fact, "absolutely ineffective," as in the particular instance on pp. 77-78, must necessarily be considered essential; and even were a treatment devised for application by practising physicians in all respects simple, it must still be thought desirable to know, from the scientific and practical standpoints, whether its curative effects are real.

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## THE WASSERMANN AND LUTIN REACTIONS IN LEPROSY.

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### INTRODUCTION.

MANY observers have reported positive results in the application of the Wassermann reaction to cases of leprosy. This investigation was undertaken to determine if the value of the reaction, as employed in this laboratory, for the diagnosis of syphilis, is discounted by the occurrence of positive reactions in lepers.

In 1906 Eitner<sup>1</sup> found that the serum of a leper examined by him deflected complement in the presence of an aqueous extract of lepromata. Subsequently he obtained the same result when he employed an alcoholic extract of guinea-pig's heart in place of the lepromatous extract.

In 1908 Wechselmann and Meier reported a case of leprosy in which the serum deflected complement in the presence of an extract of syphilitic liver, in the presence of an alcoholic extract of normal human liver, and also in the presence of an emulsion of lecithin.

In the same year, Slatineanu and Daniélopolu examined the sera of 21 lepers. They used, as their antigen, an alcoholic extract of syphilitic liver and they found that, of the 21 cases, eleven gave positive reactions.

Jundell, Almquist and Sandmann, who employed an alcoholic extract of guinea-pig's heart, reported in the same year that they had examined the sera of 22 lepers with completely positive results in only four cases.

In 1909 Ehlers and Bouret examined the sera of 44 lepers. They obtained complete inhibition of haemolysis in three cases only; of the 41 remaining, 39 of the sera produced partial inhibition and two were

<sup>1</sup> See References at the end of this paper.

negative. These observers, like Jundell and Almquist, employed an alcoholic extract of guinea-pig's heart as antigen. The sera which they investigated were obtained from lepers in the West Indies and were brought to Europe, packed in ice. Three or four months had elapsed before they could be examined, and nearly all of them had become anti-complementary.

Alberto Recio reported upon the examination of 18 lepers in Senegal by Bauer's modification of Wassermann's method. Fourteen cases with *manifestations cutanées* were investigated, with the result that only one gave a negative reaction; while, of four others with anaesthetic leprosy, two were positive and two were negative.

In 1911 H. D. Bloomberg examined the sera of 21 Filipino lepers by the original procedure of Wassermann, except as regards the antigen, which was prepared from guinea-pig's heart. Eighteen of the 21 cases gave negative reactions, and Bloomberg stated that he considered it doubtful if a positive reaction was to be obtained as a result of infection with the *Bacillus leprae*, and that he thought it was necessary to consider the possibility, not only of syphilitic taint, but also of present or antecedent framboesia.

In contrast to the above, Photinos and Michaélidès, who examined 204 patients in the leper-settlement of Spinalonga, an island off the coast of Crete, obtained a large proportion of positive results. They employed the original method of Wassermann, but used an alcoholic extract of foetal liver. Out of 104 cases of tubercular and mixed leprosy, 75 per cent. gave positive results and, of 100 anaesthetic cases, 38 per cent. were positive. They concluded that, in countries where leprosy is endemic, false conclusions may be drawn from the Wassermann reaction carried out for the diagnosis of syphilis.

Montesanto and Sotiriadès have also carried out investigations in the island of Spinalonga. They employed the modifications of Bauer and Stern in the examination of 48 patients. Their results supported those of Photinos and Michaélidès.

Howard Fox in 1910 obtained similar results. He examined 60 cases by Noguchi's method. The sera of 38 of these patients, who were suffering from tubercular leprosy, gave only seven frankly negative reactions; while in 22 anaesthetic cases the reaction was negative in 19. Fox states that in no instance was a history of syphilis obtainable, nor were luetic lesions found in any of the cases.

Rocamora examined the sera of 19 cases of leprosy with positive results in 14, in none of whom was there evidence of antecedent

syphilis. He is of opinion that the substance which fixes the complement in syphilis and in leprosy is derived from the cellular formations, which he considers bear a strong resemblance to each other, in the two diseases.

It appears that the discrepancies in the results reported by various investigators are due to two things; first to differences in the methods which they have employed, some having adopted one modification of the original Wassermann reaction and some another, and secondly, the discrepancies are due to the interpretation of the final readings, where much depends upon whether instances of partial inhibition are included among the positive results or are considered as negative.

Recently, Noguchi's luetin reaction has been applied to leprosy with results even more discordant than those obtained by the Wassermann reaction. Moses T. Clegg examined 24 lepers, none of whom showed any signs of syphilis. The Wassermann reaction was positive in 11 of them; the luetin reaction was negative in all. Schnitter examined 25 Filipino lepers, 20 of whom gave positive Wassermann reactions; but, in marked contrast to the results of Clegg, 22 of them reacted positively to injections of luetin.

#### *The Author's observations.*

Through the courtesy of Dr Glenney, Medical Officer in charge of the Leper Asylum at Kuala Lumpur, an institution containing about 300 patients, I was able to examine one hundred cases taken at random from among them. Eighty-seven of them were Chinese, 11 were Tamils, one a Eurasian and one a Malay. All, with two exceptions, were of the labouring class: 51 of them were mining coolies. Six of them had been born in the Malay States or Straits Settlements, the rest were immigrants. Most of the latter had been living in the Malay States for a considerable number of years before they became lepers. Excluding two, who were lepers when they immigrated, and three, who became lepers within one year of their arrival, the remainder had been in the country, on an average, for 11 years before they developed the disease. In none of the cases was there any room to doubt the diagnosis of leprosy, which was confirmed, in all but the most conspicuous cases, by the demonstration of Hansen's bacillus in the lesions or in the nasal mucus.

The samples of blood, for examination, were collected at this laboratory, which is within a mile of the leper asylum. The sera were



inactivated a few hours after the blood had been obtained and the tests were carried out on the following day, within 24 hours of the time at which the samples were taken.

The method adopted for the examination of the sera was that of Browning, Cruickshank and McKenzie which has been employed in this laboratory for some time and has been found to be most reliable. In place of the anti-oxhaemolytic system used by the authors of the method, an anti-human system was employed.

The results of the examination are shown in Table I. Out of the 100 lepers examined, 22 gave positive Wassermann reactions and in some of them the reactions were exceptionally powerful: for instance, the serum of Pachimuttu (No. 8, Table I) deviated no less than 58 doses of complement; an extraordinary amount for a case in which there were no signs of active syphilis. The examination of 12 of the positive and a number of the negative cases was repeated on several occasions with consistent results.

Some observers hold the opinion that the Wassermann reaction in leprosy varies according as the form of the disease from which the patient is suffering be of the tubercular or of the maculo-anaesthetic type. Other writers consider that the activity of the disease is the determining factor.

While one group of investigators have found that the Wassermann reaction is more frequently positive in tubercular and mixed leprosy than in the anaesthetic variety, others have concluded that the form of the disease is a factor of no influence in this respect.

Jundell, Almquist and Sandmann concluded, from the examination of 22 lepers, that neither the type, nor the progress, nor the duration of the disease has any influence on the reaction. Ehlers and Bouret, as a result of their examination of 44 lepers, supported this view. On the other hand, the majority of workers have found a larger proportion of positive reactions among lepers suffering from the tubercular type of the disease. Photinos and Michaélidès, who examined the sera of 204 lepers, obtained 75 per cent. of positive reactions in tubercular leprosy and 38 per cent. in the anaesthetic form. Howard Fox obtained 31 positive results in the examination of 38 lepers of the tubercular type, but only three in 22 anaesthetic cases. McIntosh and Fildes state that "the reaction is chiefly to be found in the tuberoso form."

In Table I the patients examined in this laboratory have been classified as either (*a*) tubercular or (*b*) anaesthetic. All those cases with superficial nodules have been classified as tubercular, so that all



mixed cases are included under this heading. The anaesthetic group comprises macular, mutilated and anaesthetic cases free from palpable cutaneous nodules. In the first, or tubercular group, there were 44 patients, 12 (27 %) of whom reacted positively to the Wassermann test. In the second, or anaesthetic group, which comprised 56 cases, there were 10 (18 %) positive reactions. The numbers of positive results in both the groups are so small that the difference between them might be merely a matter of chance, or it might be accounted for by reactions due to syphilis, a factor which it is impossible to exclude. Six of the twelve positive tubercular cases and seven of the ten positive anaesthetic cases admitted former syphilitic infection. If these cases be excluded, there remain six cases in the tubercular group and three cases in the anaesthetic group which gave a positive Wassermann reaction. No conclusions can be drawn from such small figures.

As regards the influence of the duration of the disease on the Wassermann reaction, the onset of leprosy is, in most cases, so insidious that it is no easy matter to ascertain the date of its commencement. Except in the case of one of the patients examined here, the only information which was available on this point was the account of his disease given by the leper himself. Relying upon this information, the average duration of the disease among the 22 lepers who reacted positively to the Wassermann test was four years and eight months, as compared with an average of three years and eight months for the 78 patients who reacted negatively. Excluding those cases which had been admitted less than one month before this examination, the average length of time since their admission to the asylum was, in the positive group, one year and ten months, and, in the negative group, one year and six months. Though the average duration of the disease was longer in the positive group, there were many very old-standing cases among those who reacted negatively; no fewer than 20 of the latter had been lepers for more than five years, while among the positive cases there were only nine who had suffered from the disease for more than three. It does not appear, then, that the mere duration of the disease is a factor which influences the Wassermann reaction.

Some observers have contended that it is only in the more advanced stages of the disease that the Wassermann reaction becomes positive. Montesanto and Sotiriadès consider that the increase of the leprosy lesions produces a greater abundance of antibodies which cause deviation of complement, and therefore that advanced cases of the disease are

more likely to give positive reactions. Among the 22 positive cases examined in this laboratory, there were several instances in which the disease was not in an advanced stage; for instance, Ah Wai (No. 35, Table I) showed nothing more than a few inconspicuous, scattered anaesthetic patches; Pachimuttu (No. 8) had similar lesions with the addition of a few minute tubercles on the ears; yet his serum deviated an exceptionally large amount of complement. Seventeen of the 22 positive cases were in an advanced and conspicuous stage of the disease, and it might be argued that the remaining five, which were cases of early or arrested leprosy, reacted positively by reason of syphilitic infection and, indeed, they all five admitted former venereal disease. On the other hand, however, there were many advanced and progressing cases among the lepers who reacted negatively; cases quite as advanced and progressing quite as rapidly as any in the positive group. As instances may be mentioned the Malay (No. 27, Table I), a case of actively progressing tubercular leprosy, and Chiew Tung (No. 34), whose face and body were covered with red, hyperaemic, active-looking tubercles. In short, the Wassermann reaction does not appear to be influenced by the duration or the activity of the disease.

In an investigation undertaken with the object of determining if leprosy *per se* can, in some cases, so modify the serum that the Wassermann reaction becomes positive, the difficulty encountered at the outset is the impossibility of excluding the disturbing factor of syphilitic infection.

As a check upon the results of the Wassermann reaction and in order to determine what proportion of the lepers examined were likely to have been infected with syphilis the following means were available:

- (1) Inspection of patients in order to determine the presence of venereal sores, scars, or the stigmata of congenital syphilis.
- (2) Interrogation of the patients.
- (3) Examination of the patients' families.
- (4) Comparison of the results of the Wassermann reaction, in the group of lepers, with the results of the reaction, as applied to a similar group of persons who were not suffering from leprosy.
- (5) Examination of the lepers for the presence of the *Treponema pallidum*.
- (6) The effect of Salvarsan upon the Wassermann reaction in those cases which reacted positively.
- (7) The luetin reaction.

As a great majority of the lepers were immigrants, the examination of the patients' families was possible in only one case. It was not considered justifiable to employ injections of Salvarsan. While this drug does not cure leprosy, its employment is by no means free from danger.

*Inspection of patients.* None of the patients was suffering from visibly active syphilis and in only one of them (No. 8, Table I) were definite syphilitic scars to be found. It is not always an easy matter to decide by inspection whether lepers, with their harsh, dry skins, macules and tubercle swellings, are free from the stigmata of syphilis or are not.

In three of the positive cases (Nos. 54, 59 and 89, Table I) the patients had been lepers since the ages of eleven, five and fifteen respectively. It is unlikely that they had acquired syphilis, and as far as could be determined, in these unfortunate boys terribly disfigured by tubercular leprosy, none of them showed any signs of congenital lues.

*Interrogation of the patients.* The information to be obtained from patients as to the occurrence of former venereal disease is notoriously unreliable. Chinese distinguish between gonorrhoea and chancres, but, to them, "syphilis" implies the manifestations of the secondary and tertiary stages. It was found that some of the patients who stated that they had suffered from syphilis had never had chancres, and on further enquiry it appears probable that they had mistaken the lesions of leprosy, first appearing, for those of syphilis.

Thirty-three of the lepers admitted former syphilis and 13 of these gave positive Wassermann reactions. Among the remaining 67 who denied former syphilis, there were nine positive reactions.

*Examination of patients' families.* As noted above, this was possible in only one case (No. 59, Table I). The mother and a young sister, aged nine, of this patient were inspected. Their serum could not be obtained for the application of the Wassermann reaction. No history was obtained from the mother which pointed to syphilitic infection and the sister showed none of the stigmata of the congenital disease.

*Comparison with a similar group of non-leprous persons.* It is interesting to compare the number of positive Wassermann reactions among the lepers here—22 out of 100—with the results obtained by Baermann and Wetter in the examination of normal coolies in Sumatra. They found that 7 per cent. of their coolies were manifestly syphilitic and that 20 per cent. gave positive Wassermann reactions; in lepers



they obtained 50 per cent. of positive results. In the Federated Malay States venereal diseases are very prevalent, so much so that, in his annual report for 1913, the Principal Medical Officer declared that they were of "universal incidence." It therefore appeared possible that all of the 22 positive Wassermann reactions, which occurred in the lepers examined here, were due to syphilis and that none of them was due to leprosy *per se*.

For purposes of comparison 110 inmates of the District Hospital at Kuala Lumpur were examined for syphilis. Forty of these people were suffering from beri-beri and 70 of them from malaria. Twenty per cent. of them either had scars on the penis or admitted that they had suffered from syphilis. In 11 of the cases, eight of whom gave a history of syphilis, the Wassermann reaction was positive. Four of the positive reactions occurred among the 40 beri-beri patients and seven among the 70 malaria patients. That is to say that the percentage of positive Wassermann reactions in the leper group was more than twice as large as that obtained in the control group of non-leprous patients. Owing to the comparatively small number of cases investigated it cannot be concluded, on these grounds alone, that the larger number obtained in the former group was due to the action of some factor other than syphilis. In the case of the lepers, where there were 22 positive reactions out of 100 cases, the "probable error" calculated by Poisson's formula is 0.11; so that, in the next hundred lepers, there might be as many as 33 or as few as 11 positive Wassermann reactions. In the control group of 110 patients, with 11 positive reactions, the probable error is 0.08, so that in another similar group of the same size the number of positive reactions might be any figure between three and 19.

*Examination for the presence of the Treponema pallidum.* It is generally agreed that, in syphilitics, the Wassermann reaction is positive only for so long as the infection continues; that is to say, a positive reaction implies the presence of the *Treponema pallidum* within the tissues. If, then, those lepers who reacted positively, did so, not because they were lepers but because they were suffering, in addition, from syphilis, it appears not unlikely that the *Treponema pallidum* might be found in the leprotic tubercles and macules, where the resistance of the tissues is diminished. Serum expressed from the depths of such lesions was examined by dark-ground illumination in 10 of the cases which reacted positively but no *Treponemata* were found.

*The luetin reaction.* Noguchi's luetin consists of killed cultures of



the *Treponema pallidum*. The use of this preparation in the diagnosis of syphilis is analogous to that of tuberculin in von Pirquet's test for tuberculosis; that is to say, if it is injected into the skin during certain stages of syphilitic infections—notably in the latent tertiary stage—it produces a cuti-reaction. The sample of luetin used in this investigation was kindly supplied by Dr Noguchi of the Rockefeller Institute, and it was thoroughly tested by its employment on the one hand in many cases of syphilis and, on the other, in cases of malaria and beri-beri.

It was considered that by the employment of luetin some light might be thrown upon the question of the reason for the positive Wassermann reaction in leprosy; whether it is always due to syphilis or whether it may be due to leprosy *per se*. Among the 22 lepers who gave a positive Wassermann reaction there were no cases of active syphilis, and if any of them were suffering from infection with that disease, it was in the latent tertiary stage; that is to say, the stage in which the luetin reaction is most often positive.

It was decided to test the effect of luetin upon (a) a group of lepers in whom the Wassermann reaction was positive, (b) a group of lepers in whom the Wassermann reaction was negative, and (c) a group of non-leprous persons among whom there were likely to be many cases of latent syphilis. As mentioned above, the sample of luetin which was used had already been tested by its employment in cases of syphilis and also in cases free from both syphilis and leprosy.

The lepers selected for the test were 21 in number; they comprised two classes; firstly, 13 in whom the Wassermann reaction was positive, and secondly a group of eight from which the possibility of syphilis was eliminated, as far as possible, by the selection of young lepers who showed no signs of congenital lues and who reacted negatively to Wassermann's test. For comparison with these lepers the next step was to obtain a number of suitable controls. Within half a mile of the leper asylum there is a Home or Infirmary for decrepit Chinese: blind, halt and maimed wrecks of humanity who are precluded from earning a livelihood by reason of their infirmities. It is probable that among these people there are many cases of latent tertiary syphilis and from among them 28 cases were selected who appeared likely to have suffered, in the past, from syphilis.

As far as the Wassermann reaction is concerned there was little difference between the lepers and the decrepits; among the 21 lepers there were 13 positive reactions and among the 28 decrepits there were

12 positive reactions. Supposing that the positive Wassermann reactions among the lepers were due to old syphilitic infections, it appeared that the group of lepers and the group of decrepits should react in the same way to inoculations of luetin. So far however was this from being the case, that, as a reference to Tables II and III will show, there was not a single positive luetin reaction among the lepers, while among the decrepits there were no fewer than 11.

The evidence of the luetin reaction as applied to these cases of leprosy is negative evidence and too much value should not be attached to it; but, as far as it goes, it is opposed to the view that positive Wassermann reactions in leprosy are due to syphilitic infection.

TABLE I.  
*Lepers examined by the Wassermann and Luetin reaction.*

Number	Name	Nation	Age and occupation	Duration of leprosy and of residence in asylum	Type	Principal features	Length of residence in Malay States	History of syphilis	Wassermann reaction	Amount of complement deviated	Luetin reaction
1	Tan Tay	Chinese (Sin Yeu)	30 Mining cooly	A leper for 1 year. In asylum for 1 month	Anaesthetic	Contractures of fingers of left hand. Not conspicuous	Not known	No history of syphilis	Negative	—	—
2	Yeo Choon	Chinese (Hokkien)	25 Mining cooly	A leper for 2 years. In asylum for 2 months	Anaesthetic	Contractures of fingers. Not conspicuous	7 years	Syphilis 3 years before	Negative	—	—
3	Dumb Boy	Chinese (Kheh)	16 nil	A leper for (?). In asylum for 2 years	Tubercular	Tubercles on face and ears. A conspicuous case	Not known	No manifest signs of syphilis	Negative	—	—
4	Ge Tiam	Chinese (Hokkien)	38 Mining cooly	A leper for 3 years. In asylum for 4 months	Tubercular	Enormous keloid-like tubercles on face. Very conspicuous	17 years	No history of syphilis	Negative	—	—
5	Konduchetty	Tamil	35 Estate cooly	A leper for 2 years. In asylum 1½ years	Anaesthetic	White anaesthetic patches on face and arms. Not very conspicuous	20 years	No history of syphilis	Negative	—	—
6	Superian	Tamil	35 Estate cooly	A leper for 4 years. In asylum 3 years	Anaesthetic	On the left arm there is an anaesthetic patch like an artificial wheal. Not conspicuous	30 years	No history of syphilis	Negative	—	—
7	Govindan	Tamil	30 Railway cooly	A leper for 2 years. In asylum 1 year	Anaesthetic	A little wasting of thenar muscles. Not conspicuous	15 years	No history of syphilis	Negative	—	—
8	Pachinuttu	Tamil	25 Railway cooly	A leper for 2½ years. In asylum 8 months	Anaesthetic	A few dried-up tubercles on the ears and some anaesthetic patches. Not conspicuous	8 years	Syphilis 3 years ago. Scar on penis	Positive on 6 occasions	58 doses	Negative
9	Ah Loong	Chinese (Hylam)	24 Estate cooly	A leper for several years. In asylum 1½ years	Anaesthetic	Amputations of toes of right foot. Conspicuous	10 years	Syphilis 2 years ago	Negative	—	—

10	Munusamy	Tamil	30 Estate coolie	A leper for 4 years. In asylum for 3 years	Anaesthetic	Right foot much swollen and anaesthetic. Not conspicuous	3 years	No history of syphilis	Negative	—
11	Punusamy	Tamil	25 Road coolie	A leper for 2 years. In asylum 1 year	Anaesthetic	Amputation of toes. Paralysis and con- tractures of fingers. A conspicuous case	10 years	No history of syphilis	Negative	—
12	Lye Poh	Chinese (Cantonese)	24 Mining coolie	A leper for 1 year. In asylum 2 months	Tubercular	Tubercles on both ears. Not a conspicuous case	6 years	No history of syphilis	Negative	—
13	Yap Slin	Chinese (Kheh)	40 Mining coolie	A leper for 10 months. In asylum 1½ months	Tubercular	Tubercles over the lower part of the face and papules on arms and trunk. Conspicuous	15 years	No history of syphilis	Negative	—
14	Phang Tuck	Chinese (Cantonese)	28 Mining coolie	A leper for several years. In asylum 2 years	Tubercular	Prominent tubercles on face and ears. Con- spicuous	10 years	Syphilis 4 years ago	Negative	—
15	Leong Sang	Chinese (Cantonese)	43 Mining coolie	A leper for 2 years. In asylum for 1½ months	Anaesthetic	Tips of ears swollen. An inconspicuous patch on right cheek	21 years	Syphilis 7 years ago	Negative	—
16	Mok Khuan	Chinese (Cantonese)	44 Mining coolie	A leper for 10 months. In asylum for 6 months	Anaesthetic	Circinate patch on right forearm and pink patches on face. Not conspicuous	4 years	No history of syphilis	Negative	—
17	Ah Fook	Chinese (Kheh)	38 Mining coolie	A leper for 2 years. In asylum 2½ months	Anaesthetic	Contracture of hands. Pink raised patches on face. Not conspicuous	16 years	No history of syphilis	Negative	—
18	Ah Yeong	Chinese (Cantonese)	40 Carpenter	A leper for 3 years. In asylum 1 month	Tubercular	Tubercles on face. A conspicuous case	20 years	No history of syphilis	Negative	—
19	Lung Yeong	Chinese (Sin Yeu)	35 Hospital servant	A leper for 2 years. In asylum 1 year	Anaesthetic	Superficial sores and peripheral neuritis.	25 years	Syphilis 3 years before	Negative	—
20	Ah Fook	Chinese (Cantonese)	56 Samsu maker	A leper for 2 years. In asylum 1 month	Anaesthetic	Paralysis of right hand. A circinate patch in- volving whole of right shoulder. A patch on face. Fairly conspicu- ous	13 years	A chancre 30 years ago	Negative	—



TABLE I—(continued).

Number	Name	Nation	Age and occupation	Duration of leprosy and of residence in asylum	Type	Principal features	Length of residence in Malay States	History of syphilis	Wassermann reaction	Amount of complement deviated	Luetin reaction
21	Ah Teck	Chinese (Hylam)	36 Gardener	A leper for 5 years. In asylum 1 year.	Tubercular	Tubercles on face and ears. Sores on feet and hands. Conspicuous	6 years	No history of syphilis	Negative	—	—
22	Yap Yoon	Chinese (Kheh)	44 Mining cooly	A leper for 2 years. In asylum 3 days	Anaesthetic	Perforating ulcer of great toe. Not conspicuous	24 years	A chancre 13 years ago	Negative	—	—
23	Vong Fah	Chinese (Kheh)	40 Mining cooly	A leper for 8 years. In asylum for 2 days	Anaesthetic	Amputations of several fingers and toes. Conspicuous	12 years	Syphilis 8 years ago	Negative	—	—
24	Hon Tiam	Chinese (Kheh)	45 Woodcutter	A leper for 2 years. In asylum for 5 months	Tubercular	Old tubercles on face. Not very conspicuous	15 years	No history of syphilis	Negative	—	—
25	Kok Yew	Chinese (Hokkien)	33 Rickshaw puller	A leper for 3 years. In asylum for 2 years	Tubercular	An old ulcerated tubercular case. Conspicuous	7 years	Had syphilis 5 years ago	Positive on 3 occasions	34 doses	Negative
26	Ah Seong	Chinese (Kheh)	42 Mining cooly	A leper for 7 years. In asylum for 1½ months	Anaesthetic	Wasting of hands. Superficial sores on legs. Not conspicuous	15 years	No history of syphilis	Negative	—	—
27	Mat	Malay	20 Gardener	A leper for 1 year. In asylum 1 month	Tubercular	Generalised tubercles. Conspicuous	Born in the State of Pahang	No history of syphilis	Negative	—	—
28	Valayan	Tamil	22 Estate cooly	A leper for 3 years. In asylum for 4 months	Anaesthetic	Some superficial ulcers on the extremities. Shrunken tubercles on the ears. Not conspicuous	13 years	No history of syphilis	Negative	—	—
29	Phan Siew	Chinese (Kheh)	66 Mining cooly	A leper for 3 years. In asylum for 1 year	Anaesthetic	One side of face paralysed. Lobes of ears slightly swollen. Not a conspicuous case	15 years	No history of syphilis	Negative	—	—
30	Cheong Yoon	Chinese (Cantonese)	35 Mining cooly	A leper for 2 years. In asylum 3 weeks	Tubercular	Old faded tubercles. Fairly conspicuous	15 years	No history of syphilis	Negative	—	—

31	Karrupan	Tamil	30 Estate coolie	A leper for 3 years. In asylum for 7 months	Tubercular	Large keloid-like tubercles on the face. Very conspicuous	9 years	No history of syphilis	Negative	—
32	Wan Ng	Chinese (Cantonese)	30 Cook	A leper for 2½ years. In asylum for 4½ months	Anaesthetic	Wasting of face and twitching of cheek muscles. Not conspicuous	11 years	Chancre 3½ years ago	Negative	—
33	Chong Geok	Chinese (Hokkien)	35 Detective	A leper for 11 months. In asylum for 10 months	Anaesthetic	Paralysis of left ulnar nerve. Not conspicuous	12 years	Syphilis 4 years ago	Positive on 2 occasions	13 doses Negative
34	Chew Tung	Chinese (Kheh)	45 Bullock-cart driver	A leper for 3 years. In asylum 2 months	Tubercular	Red and apparently active, generalised tubercles. Conspicuous	10 years	No history of syphilis	Negative	—
35	Ah Wai	Chinese (Cantonese)	30 Mining coolie	A leper for 3 years. In asylum for 6 months	Anaesthetic	A few scattered anaesthetic patches. Not conspicuous	4 years	Has had chancre and bubo	Positive on 2 occasions	8 doses Negative
36	Chean Fook	Chinese (Kheh)	28 Mining coolie	A leper for 2 months. In asylum for 2 days	Anaesthetic	A few anaesthetic patches. Not conspicuous	14 years	Chancre 7 months ago	Negative	—
37	Ah Lim	Chinese (Kheh)	28 Mining coolie	A leper for 2½ years. In asylum for 7 months	Anaesthetic	Anaesthesia on one side of face. Thumb like syringo-myelia. Not conspicuous	5 years	No history of syphilis	Negative	—
38	Thong Khong	Chinese (Kheh)	48 Mining coolie	A leper for 2½ years. In asylum for 1½ years	Anaesthetic	Contractures of left hand. Paralysis of right side of face and amputations of toes of right foot. Conspicuous	20 years	No history of syphilis but had gonorrhoea 4 years ago	Positive on 3 occasions	46 doses Negative
39	Tham Sam	Chinese (Kheh)	34 Mining coolie	A leper for 1 year. In asylum for 5 months	Anaesthetic	Sores and contractures of hands	8 years	No history of syphilis	Negative	—
40	Nasathan	Tamil	28 P.W.D. coolie	A leper for 10 months. In asylum for 3 weeks	Tubercular	A few small tubercles on ears and patches on face. Not conspicuous	3 years	No history of syphilis	Negative	—
41	Lam Vun	Chinese (Cantonese)	50 Gardener	A leper for 9 years. In asylum for 7 months	Tubercular	An old mixed case with tubercles on ears, loss of nasal cartilage and amputations of toes	11 years	No history of syphilis	Negative	—

TABLE I—(continued).

Number	Name	Nation	Age and occupation	Duration of leprosy and of residence in asylum	Type	Principal features	Length of residence in Malay States	History of syphilis	Wassermann reaction	Amount of complement deviated	Lueth reaction
42	Teo Hin	Chinese (Sin Yeu)	66 Mining cooly	A leper for 4 years. In asylum for 2 years	Anaesthetic	Cicatrised tubercles of eyebrows. Slight contracture of one little finger. Not conspicuous	22 years	No history of syphilis	Negative	—	—
43	Low Cheong Ping	Chinese (Teo Chew)	50 Mining cooly	A leper for 10 years. In asylum for 7 years	Tubercular	A very advanced case with prominent generalised tubercles	20 years	No history of syphilis	Negative	—	—
44	Leow Chong	Chinese (Kheh)	54 Mining cooly	A leper for 6 years. In asylum for 3 years	Tubercular	Conspicuous tubercles on ears and on nose	28 years	No history of syphilis	Negative	—	—
45	Teh Huat	Chinese (Hokkien)	32 Mining cooly	A leper for 3 years. In asylum for 1½ years	Tubercular	Advanced conspicuous tubercular leprosy of face with great anaemia	18 years	No history of syphilis	Negative	—	—
46	Tan Oo	Chinese (Hokkien)	61 Gardener	A leper for 3 years. In asylum for 7 months	Tubercular	Typical leonine deformity of face. Sores on extremities	25 years	No history of syphilis	Positive on 2 occasions	18 doses	Negative
47	Yap Hin	Chinese (Kheh)	40 Mining cooly	A leper for 7 months. In asylum for 4 months	Tubercular	Conspicuous tubercles all over face and chest	16 years	No history of syphilis	Negative	—	—
48	Chu Ng	Chinese (Kheh)	41 Wood-cutter	A leper for 1½ years. In asylum for 1 year	Tubercular	Typical conspicuous leontiasis	Unknown	Syphilis 1½ years ago	Negative	—	—
49	Loo Wan	Chinese (Cantonese)	42 Mining cooly	A leper for 3 years. In asylum 2½ years	Tubercular	Typical leontiasis	10 years	No history of syphilis	Negative	—	—
50	Wong Cheok	Chinese (Cantonese)	34 Mining cooly	A leper for 1½ years. In asylum 1½ years	Tubercular	Conspicuous tubercles on face. Enormous ears	Unknown	No history of syphilis	Positive on 2 occasions	28 doses	Negative
51	Yap Yean	Chinese (Kheh)	40 Estate cooly	A leper for 3 years. In asylum for 2 years	Tubercular	Conspicuous tubercles on face and ulcerated tubercles on the extremities	20 years	Syphilis 3 years ago	Positive on 2 occasions	8 doses	Negative

52	Leong Kim	Chinese (Cantonese)	45 Rickshaw puller	A leper for 11 years. In asylum for 4 years	Tubercular	Lion-like facies. An old advanced anæmic case with foul ulcers	18 years	Chancere and bubo 12 years ago	Positive on 2 occasions	13 doses	Negative
53	Piang Chye	Chinese (Cantonese)	15 Parents were hawkers	A leper for 2 years. In asylum for 1½ years	Tubercular	Conspicuous tubercles on the face. No open sores	Born in the State of Selangor	No signs of con- genital syphilis	Negative	—	Negative
54	Chow Chye	Chinese (Kheh)	14 No occupation	A leper for 3 years. In asylum for 1½ years	Tubercular	Advanced generalised conspicuous tubercles	4 years	No signs of con- genital syphilis	Positive	6 doses at least	Negative
55	Ah Mok Chye	Chinese (Kheh)	14 No occupation	A leper for 4 years. In asylum for 2 years	Tubercular	Advanced generalised conspicuous tubercles	8 years	No signs of con- genital syphilis	Negative	—	Negative
56	Ah Cheong	Chinese (Cantonese)	15 No occupation	A leper for 5 years. In asylum for 4 years	Tubercular	Advanced conspicuous anæmic tubercular case with open sores on legs	Born in the State of Selangor	No signs of con- genital syphilis	Negative	—	Negative
57	Kio	Chinese (Cantonese)	15 No occupation	How long a leper unknown. In asy- lum for 3 years	Tubercular	Conspicuous tubercles on the face, sores on the hands and feet	7 years	No signs of con- genital syphilis	Negative	—	—
58	Yam Bee	Chinese (Hokkien)	17 Schoolboy	A leper for 3½ years. In asylum for 3 years	Anæsthetic	A large cicatrised patch on left cheek which is rather conspicuous	Born in Singapore	No signs of con- genital syphilis	Negative	—	Negative
59	H. Heppon- stall	Eurasian	15 No occupation	A leper for 10 years. In asylum for 4 years	Tubercular	A very advanced tuber- cular case with open sores	Born in the State of Selangor	No signs of con- genital syphilis	Positive	8 doses at least	Negative
60	Ah Kiet	Chinese (Hylam)	22 Cakeseller	A leper for 6 years. In asylum for 4 years	Tubercular	A typical conspicuous advanced tubercular case	8 years	No signs or history of syphilis	Negative	—	Negative
61	Liew Kuee	Chinese (Kheh)	31 Mining coolly	A leper for 17 years. In asylum for 7 years	Tubercular	A very advanced old anæmic tubercular case. Conspicuous	8 years	No history of syphilis	Negative	—	Negative
62	Ah Leong	Chinese (Hokkien)	25 Nil	A leper for 5 years. In asylum for 9 months	Tubercular	Advanced generalised tubercular case with sores on feet	Born in the State of Selangor	No history of syphilis	Negative	—	Negative
63	Siaw Kim	Chinese (Kheh)	30 Mining coolly	A leper for 6 years. In asylum for 3 years	Tubercular	Advanced generalised tubercular case with abrasions on hand. Conspicuous	14 years	No history of syphilis	Negative	—	Negative



TABLE I—(continued).

Number	Name	Nation	Age and occupation	Duration of leprosy and of residence in asylum	Type	Principal features	Length of residence in Malay States	History of syphilis	Wassermann reaction	Amount of complement deviated	Laethm reaction
64	Lee Pin	Chinese (Kheh)	26 Wood engraver	A leper for 4 years. In asylum for 1 year	Anaesthetic	Sores on feet. Not conspicuous	8 years	No history of syphilis	Positive on 2 occasions	8 doses	Negative
65	Ving Hoy	Chinese (Kheh)	40 Mining cooly	A leper for 3 years. In asylum for 3 years	Tubercular	Large conspicuous tubercles on the ears	16 years	Had syphilis 10 years ago	Positive	8 doses	Negative
66	Liaw Choy	Chinese (Kheh)	40 Mining cooly	A leper for more than 1 year. In asylum for 5 months	Anaesthetic	Amputations of toes. Conspicuous	10 years	No history of syphilis	Positive	3 doses	—
67	Lim Hin	Chinese (Hokkien)	26 Mining cooly	A leper for 3 years. In asylum for 2 years	Tubercular	Large conspicuous tubercles on face. Ulcers on feet	17 years	No history of syphilis	Negative	—	—
68	Cheaw Fook	Chinese (Kheh)	39 Mining cooly	A leper for 4 months. In asylum for 2 months	Anaesthetic	Red circinate erythematous patches. Not conspicuous	14 years	No history of syphilis	Negative	—	—
69	Ng Chia	Chinese (Kheh)	47 Mining cooly	A leper for 2 months. In asylum for 1 week	Anaesthetic	Amputations of ends of fingers of left hand. Not conspicuous	16 years	Had a chancre 14 years ago	Negative	—	—
70	Nadasan	Tamil	15 Estate cooly	A leper for 7 years. In asylum for 3 days	Anaesthetic	Small raised patches on arms and small ulcerated tubercles on ears. Not conspicuous	5 years	No history or sign of syphilis	Negative	—	—
71	Lye Yew	Chinese (Kheh)	34 Mining cooly	A leper for 1½ years. In asylum for 7 months	Anaesthetic	Inconspicuous reddish patches on face	8 years	No history of syphilis	Negative	—	—
72	Loong Paw	Chinese (Kheh)	27 Bootmaker	A leper for 1 year. In asylum for 1 year	Anaesthetic	A small nodule on one ear and a pale swelling on one cheek. Inconspicuous	13 months	No history of syphilis	Negative	—	—
73	Leong Yit	Chinese (Cantonese)	53 Barber	A leper for 5 years. In asylum for 3 days	Tubercular	Large pale tubercle on right ear. Inconspicuous	9 years	Syphilis about 20 years ago	Positive on 2 occasions	8 doses	—

74	Vong Fook	Chinese (Kheh)	40 Not known	Not known. In asy- lum for 1 year	Anaesthetic	Paralysis left face. In- conspicuous	Unknown. Patient is very deaf	No signs of syphilis	Negative	—
75	Chung Man	Chinese (Kheh)	49 Mining cooly	A leper for 6 years. In asylum for 4 years	Anaesthetic	Necrosis of right foot	28 years	Chancre 18 years ago	Negative	—
76	Kan Man	Chinese (Cantonese)	38 Mining cooly	A leper for 3 years. In asylum for 3 months	Anaesthetic	Small perforating ulcer. Very anæmic. Atro- phied tubercles of ears. Not conspicuous	26 years	No history of syphilis	Negative	—
77	Choo Piang	Chinese (Kheh)	45 Mining cooly	A leper for 12 years. In asylum for 6 years	Anaesthetic	Extensive amputations of fingers and toes. No tubercles	16 years	No history of syphilis	Negative	—
78	Vong Fah	Chinese (Kheh)	45 Mining cooly	A leper for 6 months. In asylum for 1 month	Tubercular	Conspicuous tubercles on the face and a few small urticarial-like patches on the trunk	20 years	No history of syphilis	Negative	—
79	Vong Chin	Chinese (Kheh)	55 Mining cooly	A leper for 6 years. In asylum for 4 years	Anaesthetic	Slight contractures of the little fingers. For- merly had superficial sores on toes. Very inconspicuous	35 years	Syphilis 8 years ago	Negative	—
80	Sin Yong	Chinese (Kheh)	45 Mining cooly	A leper for 6 years. In asylum for 8 months	Anaesthetic	Contractures of fingers and a perforating ulcer of the foot	8 years	Syphilis 6 months ago	Positive	3 doses
81	Lie Tong	Chinese (Sin Yeu)	46 Mining cooly	A leper for 2 years. In asylum for 1½ years	Anaesthetic	An inconspicuous pink- ish patch on the face	20 years	Chancre and bubo 7 years ago	Positive	6 doses
82	Liew Hin	Chinese (Kheh)	49 Mining cooly	A leper for 2½ years. In asylum for 1 year	Tubercular	Old generalised conspi- cuous tubercles	25 years	Syphilis 4 years ago	Positive on 2 occasions	8 doses
83	Liew On	Chinese (Kheh)	50 Mining cooly	A leper for 11 years. In asylum for 7 years	Anaesthetic	Conspicuous contrac- tures and amputa- tions. Perforating ulcers	32 years	A chancre 14 years ago	Positive	8 doses
84	Ong Piang	Chinese (Hokkien)	34 Gardener	A leper for 8 years. In asylum for 4 years	Tubercular	Face covered with tuber- cles. Sores on hands and feet. Conspicuous	13 years	No history of syphilis or other venereal disease	Positive	8 doses

TABLE I—(continued).

Number	Name	Nation	Age and occupation	Duration of leprosy and of residence in asylum	Type	Principal features	Length of residence in Malay States	History of syphilis	Wassermann reaction	Amount of complement deviated	Luedin reaction
85	Liew See	Chinese (Kheh)	31 Mining cooly	A leper for 2 years. In asylum for 2 years	Tubercular	Conspicuous old tubercles on face. Small sores on feet	4 years	Syphilis 2½ years ago	Negative	—	—
86	Ah Loong	Chinese (Hokkien)	30 Gardener	A leper for 7 years. In asylum for 3 years	Anaesthetic	Some faded patches on face. Chilblain-like swelling of fingers. Not conspicuous	11 years	No history of syphilis	Negative	—	—
87	Yong Fat	Chinese (Kao Chow)	39 Mining cooly	A leper for 11 years. In asylum for 1 year	Anaesthetic	White anaesthetic inconspicuous patches	20 years	No history of syphilis	Negative	—	—
88	Chin Kong	Chinese (Tco Chew)	25 Grainshop cooly	A leper for 3 years. In asylum for 1 year	Anaesthetic	Wasting of right hand	8 years	No history of syphilis	Negative	—	—
89	Siaw Seong	Chinese (Kheh)	22 Mining cooly	A leper for 7 years. In asylum for 4 months	Tubercular	Old small tubercles of ears and face. Contracture of left little finger. Conspicuous	7 years	No history of syphilis	Positive	6 doses at least	—
90	Chong Piang	Chinese (Kheh)	31 Attap house builder	A leper for 1½ years. In asylum for 1 month	Anaesthetic	Early perforating ulcer. An inconspicuous case	9 years	Syphilis 2½ years ago	Negative	—	—
91	Vong Yewn	Chinese (Kheh)	62 Goldsmith	A leper for 13 years. In asylum for 1 year	Anaesthetic	One hand has the same appearance as in syngo-myelia. Not conspicuous	40 years	No history of syphilis	Negative	—	—
92	Sim Yoong	Chinese (Kheh)	45 Mining cooly	A leper for 10 years. In asylum for 1 year	Anaesthetic	Contractures of finger of left hand. Paralysis upper left face. Sores on feet. Conspicuous	25 years	Syphilis 7 years ago	Positive	6 doses at least	—

93	Vong Ngit	Chinese (Kheh)	46 Mining cooly	A leper for 4 years. In asylum for 1 day	Anaesthetic	A few red papules on face. Red wheals on right hip. Not con- spicuous	10 years	Had syphilis 5 months ago. On being questioned further the pa- tient said that the disease con- sisted of an erup- tion on the face. It was possibly leprotic	Negative	—
94	Lee Chung	Chinese (Cantonese)	50 Mining cooly	A leper for 10 years. In asylum for 1 month	Anaesthetic	Old contractures and amputations of both hands and one foot. No sores or tubercles	23 years	Had syphilis 10 years ago	Negative	—
95	Lee Loong	Chinese (Hylam)	26 Estate cooly	A leper for 2½ years. In asylum for 1 year	Tubercular	Old tubercles on face, flattened bridge of nose and amputation of one toe. Conspicuous	10 years	Had syphilis 5 years ago	Negative	—
96	Rengasamy	Tamil	28 Railway cooly	A leper for 2½ years. In asylum for 1½ years	Anaesthetic	Wasting and contrac- tures of left hand. Cir- cinate patches on fore- head and left arm. Conspicuous	13 years	No history of syphilis	Negative	—
97	Chong Nghee	Chinese (Kheh)	48 Mining cooly	A leper for 5 years. In asylum for 10 days	Anaesthetic	Wasting and contrac- tures of left hand. Not conspicuous	12 years	Chancere and bubo 5 years ago	Negative	—
98	Lye Yoon	Chinese (Kheh)	58 Bullock- cart driver	A leper for 2 years. In asylum for 10 months	Anaesthetic	Slight contractures of fingers. Scars of healed ulcers. Not conspi- cuous	21 years	No history of syphilis	Negative	—
99	Chong Swi	Chinese (Kheh)	31 Mining cooly	A leper for 1 year. In asylum for 6 months	Anaesthetic	Perforating ulcer right foot and contractures of left hand. Con- spicuous	7 years	No history of syphilis	Negative	—
100	Lumatic	Chinese (Kheh)	30 (?) Nil	Leper for an un- known period. In asylum for 3 years	Anaesthetic	Wasting and contrac- tures of right hand and left foot. Flat- tening of bridge of nose. Conspicuous	Unknown	Unknown	Negative	—



TABLE II.

*Group of Lepers inoculated with Luetin.*

Number in Table I	Name and age		Type of Leprosy	Wassermann reaction	Luetin reaction
8	Pachimuttu	25	Anaesthetic	Positive	Negative
25	Kok Yew	33	Tubercular	Positive	Negative
33	Chong Geok	35	Anaesthetic	Positive	Negative
35	Ah Wai	30	Anaesthetic	Positive	Negative
38	Thong Kong	48	Anaesthetic	Positive	Negative
46	Tan Oo	61	Tubercular	Positive	Negative
50	Wong Cheok	34	Tubercular	Positive	Negative
51	Yap Yean	40	Tubercular	Positive	Negative
52	Leong Kim	45	Tubercular	Positive	Negative
54	Chow Chye	14	Tubercular	Positive	Negative
59	H. H.	15	Tubercular	Positive	Negative
64	Lee Pin	26	Anaesthetic	Positive	Negative
65	Ving Hoy	40	Tubercular	Positive	Negative
53	Piang Chye	15	Tubercular	Negative	Negative
55	Ah Mok Chye	14	Tubercular	Negative	Negative
56	Ah Cheong	15	Tubercular	Negative	Negative
58	Yam Bee	17	Anaesthetic	Negative	Negative
60	Ah Kiet	22	Tubercular	Negative	Negative
61	Liew Kwee	31	Tubercular	Negative	Negative
62	Ah Leong	25	Tubercular	Negative	Negative
63	Siaw Kim	30	Tubercular	Negative	Negative

TABLE III.

*Control group of decrepits inoculated with Luetin.*

Number	Name and age		Disability	History of syphilis	Wassermann reaction	Luetic reaction
1	Chua Leong	56	Blind from old ophthalmia	No history of syphilis	Positive	Positive Papular
2	Chong Yew	53	Optic atrophy	No history of syphilis	Positive	Positive Pustular
3	Chay Yeong	39	Leg amputated for necrosis of bone 8 years ago. Old scars which look syphilitic	Syphilis many years ago	Positive	Positive Pustular
4	Chin Ghan	36	Tabes and optic atrophy. Old scars which look syphilitic	No history of syphilis	Positive	Positive Pustular
7	Kuan Lin	44	Leg amputated 11 years ago for ulcers	Had a chancre a year before ulceration of leg commenced	Positive	Positive Papular
8	Koh Chan	35	Myelitis for 6 years	Had syphilis about 6 years ago	Positive	Negative

TABLE III—(continued).

*Control group of decrepits inoculated with Luetin.*

Number	Name and age	Disability	History of syphilis	Wassermann reaction	Luetin reaction
9	Chin Siew	60 Optic neuritis	? (Stone deaf)	Positive	Negative
10	Chea Thean	60 Blind for about 14 years from plastic iritis	Had syphilis about 14 years ago	Positive	Negative
11	Foo Lian	56 Blind for about 10 years from plastic iritis	No history of syphilis	Positive	Negative
12	Liew Yai	49 Tabes and optic atrophy	Had syphilis 20 years ago	Positive	Negative
13	Chong Fat	43 Optic atrophy	No history of syphilis	Positive	Negative
14	Lin Sang	50 Contractures and scars 8 years	No history of syphilis	Positive	Negative
15	Chin Sang	38 Blind from old ophthalmia for 10 years	27 years ago	Negative	Positive Papular
16	Foong Wey	38 Blind from old ophthalmia	No history of syphilis	Negative	Positive Pustular
17	Phung Nghee	58 Blind from old ophthalmia	No history of syphilis	Negative	Positive Torpid
18	Sia Tiap	43 Leg amputated for bone necrosis	25 years ago	Negative	Positive Pustular
19	Chin Yoon	35 Leg amputated for ulcers 10 years ago. Many scars apparently syphilitic	Denies syphilis but admits bubo years ago	Negative	Positive Pustular
20	Liew Fook	48 Optic atrophy. Many scars which look syphilitic	No history of syphilis	Negative	Positive Pustular
21	Ngai Siew	32 Optic atrophy	No history of syphilis	Negative	Negative
22	Lye Song	63 Myelitis 15 years	24 years ago	Negative	Negative
23	Lin Choon	35 Myelitis 3 years	No history of syphilis	Negative	Negative
24	Kum Seong	35 Leg amputated 3 years ago for ulceration	No history of syphilis	Negative	Negative
25	Chong Kiew	36 Leg amputated for ulceration 1 year ago	No history of syphilis	Negative	Negative
26	Tai Chon	33 Leg amputated 2½ years ago after an accident	No history of syphilis	Negative	Negative
27	Lee Yoon	56 Blind for 20 years from old ophthalmia	No history of syphilis	Negative	Negative
28	Liew Kwee	58 Optic atrophy. Blind for 5 years	No history of syphilis	Negative	Negative
29	Cheng Swee	37 Myelitis of 3 years' standing	No history of syphilis	Negative	Negative
30	Goh Lian	50 Leg amputated 3 years ago for ulcers	No history of syphilis	Negative	Negative

## SUMMARY.

1. One hundred lepers were examined by Browning, Cruickshank and McKenzie's modification of the Wassermann reaction with positive results in 22 cases. The amount of complement deviated was in some cases exceptionally large.

2. In a control group of 110 non-leprous persons there were 11 positive reactions.

3. In only one of the lepers was there visible evidence of former venereal disease but 33 of the lepers admitted that they had suffered from syphilis or from chancres, and of these 33, 13 reacted positively, while only nine of the remaining 66 gave positive reactions.

4. In the control group, 21 admitted former syphilitic infection and, of these, eight reacted positively; among the remaining 89, who denied syphilis, there were three positive reactions.

5. There were 12 positive reactions among 44 cases of tubercular leprosy and 10 positive reactions among 56 cases of the anaesthetic type.

6. The average duration of the disease, among the 22 lepers who reacted positively, was four years and eight months, as compared with an average of three years and eight months for the 78 patients who reacted negatively; but among the latter there were many old-standing cases.

7. Seventeen of the 22 positive cases were in an advanced stage of leprosy and in some of them the disease was progressing; but among those lepers who reacted negatively there were also many advanced and progressing cases.

8. Serum from the lesions in 10 of the lepers who reacted positively was examined by dark-ground illumination, but in no case was the *Treponema pallidum* found.

9. The luctin test was applied to 21 lepers, with negative results in every instance; 13 of these cases gave positive and eight gave negative Wassermann reactions. The test was also applied to a control group of non-leprous persons selected because they were likely to be latent syphilitics. In this group there were 11 positive luetin reactions.

*The conclusions to be drawn from the results.*

There were no clinical criteria by which one could foretell the results of the application of the Wassermann test to the lepers who were examined, and, if it be admitted that the positive reactions were due to leprosy and not to syphilis, it is difficult to understand why some tubercular and some anaesthetic cases reacted positively while others, clinically similar, did not; or why some actively, progressing cases reacted positively while others as active and as progressive gave negative reactions; but because the determining factor in these reactions has not been demonstrated it must not be concluded that this is necessarily latent syphilis.

The number of positive reactions in the group of lepers was double that which occurred in the control group; but in the former a larger number of individuals admitted antecedent syphilitic disease.

A striking feature, in some instances, was the strength of the positive reactions given by the leper sera tested, not once only, but on several occasions. The deviation of complement in such amounts as 50 or 30 doses is, at least, a rare occurrence in latent syphilis.

The negative results of the luetin test and of the search for *Treponema* are in favour of the view that the positive Wassermann reactions were due to some other cause than syphilis.

On the whole it appears probable from the results of this investigation that leprosy, apart from syphilis, may cause a positive deviation of complement when the serum is examined by the method of Browning, Cruickshank and McKenzie.

Leprosy does not cause the luetin reaction to become positive.

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# THE INFLUENCE OF AGE OF THE GRAND-PARENT AT THE BIRTH OF THE PARENT ON THE NUMBER OF CHILDREN BORN AND THEIR SEX.

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(With 1 Text-figure.)

It has been already shown by Darwin and others that reproduction is a function of environment and that unfavourable conditions of life are likely to increase the number of seeds formed or offspring produced; hence so many are reproduced that the chance of the species being perpetuated is maintained, despite the unfavourable conditions for survival. To extend this idea, and say that a dying species tends to reproduce faster than one actively evolving, is a suggestion worthy of enquiry. It has been argued that the varying birth rates in man may to some extent reflect the future biological possibilities of his race. In so far as all environments are really functions of time, or, to put it another way, we measure the nature of an environment by the length of time it takes to produce a certain result, it is quite a natural sequence to consider the effect of time, that is, age on fertility. Dr Matthews Duncan and others have dealt with the immediate effect of time, that is personal age, and the question of the transmitted effect, if any, remains to be considered. If the idea that a species which is dying out tends to reproduce more rapidly, holds good for man, then our previous finding, that the later born do not on the average live so long as the earlier born, would suggest that they might possess as compensation an enhanced fertility. To solve this problem we must correlate the number of offspring produced by each unit with the age of the parents when he or she was born, the reproductive period being made constant. It is obvious that considerable difficulties will arise owing to the imperfect nature of our data, for we should possess knowledge not only of the number born alive, but also of still births and miscarriages. Should the latter not be recorded, a negative correlation is to be expected, if our

previous findings are correct, for it will be remembered that the correlation between age of grandmother at the birth of the mother and the number dying in adolescent life, is about .1 and further, that this association is more marked in early life. An idea of the value may be deduced in the following way. If we denote by  $X_1$  the number of live births, by  $X_2$  the number of still births, and by  $X_3$  the grandmother's age at the birth of the mother, then the correlation of  $X_3$  age and  $X_1 + X_2$  (the total number of births) is

$$\frac{r_{13}\sigma_1\sigma_3 + r_{23}\sigma_2\sigma_3}{\sigma_3(\sigma_1^2 + \sigma_2^2 + 2r_{12}\sigma_1\sigma_2)^{\frac{1}{2}}}.$$

If  $r_{23}$  is positive, the expression will only vanish if  $r_{13}$  is negative.

With these preliminary remarks, we may turn to the actual data. The information on which the following work is based was obtained from Burke's *Peerage*, 1902, and deals with all those who actually inherited a peerage; the other members of the family are ignored. The following points deserve consideration: (1) only such members of the family as had actually been christened are given. Hence still births or such as died shortly afterwards would not be recorded. It is also probable that those who died in infancy are not fully accounted for. If the record is comprehensive, then the rate of infant mortality in the peerage must be under 25 per 1000 births, a somewhat unlikely figure. It is obvious that this omission will produce an inaccuracy in the actual size of the family and, as has already been stated, may tend to produce a negative correlation in the data. (2) In so far as we were dealing with males only, and some had been married twice, it was decided to count each of the latter from his first marriage to the death of his first wife and again from his second marriage to his own death or the death of his second wife and so on. (3) About 20 per cent. of those considered are still living. (4) 75 per cent. are first male births. This proportion is so large that it may appear unnecessary to consider order of birth, but, as will be seen later, some weight had to be given to it. (5) Instances where no marriage occurred are ignored.

The characters observed and the reason for choosing each were as follows:

1. Age of father at birth of peer.
2. Number of children born to peer. (These are fundamental and need no further consideration.)
3. Age of peer at marriage.
4. Age of peer at death or present age.

These are necessary because the actual space of time during which reproduction occurs must be made constant. It is unfortunate that the age of the wife was unobtainable as it is obvious that in many cases where the wife was still living, the reproductive epoch had ended before the husband's death. An attempt has been made to remove this difficulty in the second series of observations.

### 5. Order of male births.

It would have been better to have known the actual birth sequence, but this is not given. Even should we decide to ignore the possible pathological handicap of the first born as a statistical fallacy, we must still give order of birth some consideration, because the first male stands in a somewhat different position from that of other members of the family. He has everything to gain by a large family, with respect to the perpetuation of his house, whilst his brothers are in a position more akin to that of the prosperous middle class, where the reverse holds good, so that should a peerage fall to a later born child, it might wholly change his views respecting his responsibilities. Such a possibility renders it desirable that order should be considered. The tables formed are as follows:

TABLE I.

*Age of father at birth of son and number of children born to son.*

Age of father at birth	Years	Number born																		Totals
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
15-16	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	2
17-18	—	—	1	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	3
19-20	—	—	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	3
21-22	6	6	4	—	3	6	3	2	1	1	1	—	—	—	—	—	—	—	—	33
23-24	10	7	6	8	7	13	4	5	4	3	1	3	2	—	1	—	1	—	—	75
25-26	17	1	8	9	5	9	10	8	5	8	8	2	3	2	—	—	—	—	—	95
27-28	17	6	8	12	13	15	13	6	9	5	5	3	5	1	2	—	—	—	—	120
29-30	14	9	7	13	13	8	16	9	4	5	2	4	1	1	1	1	1	—	1	110
31-32	17	12	6	15	12	9	14	10	7	5	2	4	3	—	4	—	—	—	—	120
33-34	11	6	8	9	6	3	10	12	7	10	5	3	1	2	1	—	—	—	—	94
35-36	6	5	9	12	9	10	9	6	6	2	4	4	2	1	2	—	—	—	—	87
37-38	12	3	5	12	6	14	5	7	5	5	3	3	1	—	—	—	—	—	—	81
39-40	9	4	5	1	10	9	9	5	3	6	3	1	—	—	2	—	—	—	—	67
41-42	8	6	3	4	9	5	4	3	4	2	1	1	1	—	—	—	—	—	—	51
43-44	7	2	2	2	3	3	7	5	2	2	—	—	—	—	—	—	—	—	—	35
45-46	2	3	3	4	2	2	4	1	1	3	1	1	2	—	—	—	—	—	—	29
47-48	2	3	1	—	3	2	1	1	—	—	—	2	1	—	1	1	—	—	—	18
49-50	2	3	1	2	1	—	—	—	1	1	1	1	—	—	1	—	—	—	—	14
51-52	6	1	1	2	—	2	1	1	2	—	—	—	1	—	—	—	—	—	—	17
53-54	3	—	—	2	2	—	—	—	—	—	—	—	3	—	—	—	—	—	—	9
55-56	2	1	—	1	—	—	1	—	—	—	—	—	1	1	—	—	—	—	—	6
57-58	2	1	—	1	—	—	—	1	2	—	—	—	—	1	—	—	—	—	—	7
59-60	1	2	1	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	7
61-62	1	—	—	—	—	—	1	—	—	—	—	1	—	—	—	—	—	—	—	2
63-64	—	—	2	—	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	4
65-66	—	—	—	—	—	2	—	—	—	1	—	—	—	—	1	—	—	—	—	4
67-68	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
69-70	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
71-72	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
73-74	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
75-76	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
77-78	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
79-80	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1
Totals	155	82	81	111	106	117	112	85	63	59	38	33	25	9	15	2	2	—	1	1096

All  $\sigma_{\text{no. born}} = 3.5946$ .  $\sigma_{\text{age at birth}} = 4.3858$ .  $r = -.0203 \pm .0198$ .

If sterile mating be ignored  $r = -.0122 \pm .0203$ .  $\eta_{\text{age}} = .1943 \pm .0196$ .  $\eta_{\text{number born}} = .0882 \pm .0202$ .





TABLE III.

*Age of father at birth of son and age of son at marriage.*

Age at birth		Age of son at marriage																				Totals																
Years		13-14	15-16	17-18	19-20	21-22	23-24	25-26	27-28	29-30	31-32	33-34	35-36	37-38	39-40	41-42	43-44	45-46	47-48	49-50	51-52	53-54	55-56	57-58	59-60	61-62	63-64	65-66	67-68	69-70	71-72	73-74	75-76	77-78	79-80			
15-16		1					1				1																											
17-18							1	1			1	1	1	1																								
19-20							2	4			2	3	3	3																								
21-22				1	1	7	14	6	11	7	6	2	4	3	2																							
23-24	1		1	1	2	10	20	11	17	9	8	5	3	3	3	2																						
25-26					4	18	19	14	11	10	11	7	6	4	3	2																						
27-28					2	17	16	15	15	9	8	6	4	5	4	2																						
29-30				2	4	22	28	10	15	8	11	5	4	4	2																							
31-32				2	13	16	22	9	8	7	13	8	6	4	2																							
33-34				2	13	9	8	13	12	9	5	10	6	4	2																							
35-36				3	6	14	9	5	7	10	4	3	3	3	2																							
37-38				2	5	8	10	2	9	4	3	3	3	3	2																							
39-40				1	5	3	6	3	3	2	4	3	1	2																								
41-42					1	3	3	3	3	2	1	3	1	2																								
43-44						1	2	1	1	3	1	1	1	1																								
45-46							1	1	1	1	1	1	1	1																								
47-48							2	1	3	1	1	1	1	1																								
49-50							1	3	1	3	1	1	1	1																								
51-52							5	2	1	1	1	1	1	1																								
53-54							1	1	1	2	1	1	1	1																								
55-56																																						
57-58							1	1	1																													
59-60								1	2																													
61-62									1																													
63-64																																						
65-66																																						
67-68																																						
69-70																																						
71-72																																						
73-74																																						
75-76																																						
77-78																																						
79-80																																						
Totals		1	2	5	31	132	168	157	125	106	84	70	48	45	27	22	18	10	9	9	6	3	3	3	1	3	2	1	1	1	1	1	1	1	1	1092		

 $\sigma_{\text{age at birth}} = 4.7216, \quad \sigma_{\text{age at marriage}} = 3.9039, \quad r = -.0398 \pm .0194.$

TABLE IV.

*Age of son at marriage and age of son at death.*

[illegible]

TABLE V.

*Age of son at marriage and number of offspring born (all births).*

Years	13-14	15-16	17-18	19-20	21-22	23-24	25-26	27-28	29-30	31-32	33-34	35-36	37-38	39-40	41-42	43-44	45-46	47-48	49-50	51-52	53-54	55-56	57-58	59-60	61-62	63-64	65-66	67-68	69-70	71-72	73-74	75-76	77-78	79-80	Totals	
0	—	—	—	—	—	17	17	18	14	15	9	7	12	3	4	4	3	1	2	4	1	1	—	—	—	—	—	—	—	—	—	—	—	—	1	154
1	—	—	—	4	10	15	6	4	7	8	3	3	5	2	3	2	4	3	3	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	81
2	—	—	—	2	9	12	13	11	8	8	10	9	3	4	5	2	—	1	1	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	81
3	—	—	1	2	9	12	19	12	8	8	10	9	4	5	5	1	1	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	106	
4	1	1	2	2	14	17	13	9	11	8	13	10	5	5	2	1	1	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	117	
5	—	—	—	2	9	20	14	18	13	11	7	2	2	3	2	1	—	—	3	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	112	
6	—	—	—	3	16	14	15	23	11	10	10	9	1	3	1	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	84	
7	—	—	—	3	14	17	11	4	5	8	6	3	4	3	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	58	
8	—	—	1	3	6	11	10	5	8	4	3	3	3	1	1	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	59	
9	—	—	—	2	7	9	7	4	4	1	1	2	4	1	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	39	
10	—	—	—	2	5	6	4	3	6	1	1	2	1	2	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	36	
11	—	—	—	2	5	6	6	3	4	4	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	32	
12	—	—	—	—	1	4	1	3	2	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	25	
13	—	—	—	—	3	4	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	9	
14	—	—	—	—	—	2	1	1	1	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	15	
15	—	1	—	—	3	—	1	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	
16	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	
17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	
18	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	
Totals	1	2	5	31	132	166	152	130	105	84	68	50	44	27	22	18	10	9	9	6	3	4	3	3	1	3	1	—	—	2	—	—	—	—	1098	

 $\sigma_{\text{no. born}} = 3.5992$ .  $\sigma_{\text{age at marriage}} = 3.9069$ ,  $r = -.2126 \pm .0159$ .



TABLE VI.

*Age at death of son and number born to son.*

Years	Number born																		Totals	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		18
21-22	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
23-24	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3
25-26	3	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
27-28	2	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
29-30	4	3	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8
31-32	5	2	1	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	11
33-34	3	3	3	1	2	3	—	—	—	—	—	—	—	—	—	—	—	—	—	15
35-36	5	4	4	3	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	19
37-38	10	3	3	3	1	2	2	—	—	—	—	—	—	—	—	—	—	—	—	24
39-40	4	4	2	5	3	6	4	—	—	1	—	—	—	—	—	—	—	—	—	29
41-42	9	2	2	1	4	4	3	1	—	—	—	—	—	—	—	—	—	—	—	26
43-44	4	3	3	5	4	1	4	2	2	—	2	—	—	—	—	—	—	—	—	30
45-46	2	3	8	3	2	3	5	1	—	1	2	1	—	1	—	1	—	—	—	32
47-48	4	4	3	7	4	4	2	4	2	3	—	—	1	—	1	—	—	—	—	39
49-50	10	4	4	5	5	3	6	2	—	2	1	1	1	—	—	—	—	—	—	44
51-52	5	3	1	6	5	4	5	2	1	2	1	—	1	1	—	—	—	—	—	37
53-54	8	2	4	7	6	5	2	1	1	1	2	2	—	—	—	—	—	—	—	41
55-56	4	4	7	3	3	3	6	2	2	1	2	2	3	—	—	—	—	—	1	42
57-58	5	4	2	2	5	5	1	5	4	1	1	3	1	—	1	—	—	—	—	40
59-60	6	2	2	3	3	6	4	3	2	4	2	2	2	—	1	—	—	—	—	42
61-62	3	3	5	11	7	5	5	7	1	4	4	3	—	1	1	—	—	—	—	60
63-64	3	2	3	8	6	9	3	5	1	2	—	1	1	—	1	—	—	—	—	45
65-66	5	1	3	1	4	7	5	4	4	1	4	—	2	—	—	—	—	—	—	42
67-68	9	1	5	3	1	6	5	4	1	3	1	1	1	2	1	—	—	—	—	44
69-70	3	2	1	6	9	4	5	2	6	6	4	1	1	—	1	—	2	—	—	53
71-72	11	2	—	4	1	3	4	9	6	2	4	3	1	1	2	—	—	—	—	53
73-74	6	4	2	2	4	5	4	7	4	6	1	2	3	—	—	—	—	—	—	50
75-76	6	4	3	3	4	6	6	7	2	3	3	6	4	—	2	—	—	—	—	59
77-78	2	1	1	2	4	2	1	6	9	5	1	1	1	—	—	—	—	—	—	36
79-80	3	4	4	1	3	4	5	2	2	—	2	1	—	—	1	1	—	—	—	33
81-82	1	1	2	4	3	5	4	1	3	6	—	1	1	1	—	—	—	—	—	33
83-84	2	1	2	2	4	6	7	2	—	2	1	—	—	—	1	—	—	—	—	30
85-86	1	1	—	3	3	3	3	2	3	1	—	1	—	—	1	1	—	—	—	23
87-88	2	—	—	1	2	—	4	2	3	2	—	—	—	2	—	—	—	—	—	18
89-90	1	—	—	—	—	1	3	1	—	1	1	—	—	—	—	—	—	—	—	8
91-92	1	1	—	—	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	5
93-94	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
95-96	—	—	—	—	1	—	—	1	1	—	—	—	—	—	—	—	—	—	—	3
97-98	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
99-100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
101 and over	—	—	—	1	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	3
Totals	155	82	81	110	106	119	119	85	60	59	38	33	26	8	15	2	2	—	1	1091

$$\sigma_{\text{no. born}} = 3.5992. \quad \sigma_{\text{death}} = 7.7560. \quad r = .3174 \pm .0138.$$

TABLE VII.

*Order of birth and age at death.*

Years	Order								Totals
	1st	2nd	3rd	4th	5th	6th	7th	8th	
21-22	1	—	—	—	—	—	—	—	1
23-24	2	—	—	1	—	—	—	—	3
25-26	2	3	—	—	—	—	—	—	5
27-28	5	—	—	—	—	—	—	—	5
29-30	6	—	2	—	—	—	—	—	8
31-32	10	1	—	—	—	—	—	—	11
33-34	12	2	1	—	—	—	—	—	15
35-36	18	1	—	—	—	—	—	—	19
37-38	18	4	2	—	—	—	—	—	24
39-40	24	4	1	—	—	—	—	—	29
41-42	21	5	—	—	—	—	—	—	26
43-44	28	2	—	—	—	—	—	—	30
45-46	24	8	—	—	—	—	—	—	32
47-48	32	4	3	—	—	—	—	—	39
49-50	39	3	2	—	—	—	—	—	44
51-52	29	6	2	—	—	—	—	—	37
53-54	31	9	1	—	—	—	—	—	41
55-56	34	6	2	—	—	—	—	—	42
57-58	35	5	1	—	—	—	—	—	41
59-60	37	5	—	—	—	—	—	—	42
61-62	51	7	2	—	—	—	—	—	60
63-64	34	6	4	1	—	—	—	—	45
65-66	36	5	1	—	—	—	—	1	42
67-68	38	5	1	—	—	—	—	—	44
69-70	40	9	3	1	—	—	—	—	53
71-72	43	8	1	1	—	—	—	—	53
73-74	39	6	4	—	1	—	—	—	50
75-76	48	8	1	2	—	—	—	—	59
77-78	26	9	1	—	—	—	—	—	36
79-80	25	4	4	—	—	—	—	—	33
81-82	20	7	3	1	—	1	—	1	33
83-84	24	5	1	—	—	—	—	—	30
85-86	19	1	1	2	—	—	—	—	23
87-88	12	3	2	1	—	—	—	—	18
89-90	6	2	—	—	—	—	—	—	8
91-92	3	—	2	—	—	—	—	—	5
93-94	2	1	—	—	—	—	—	—	3
95-96	—	—	—	—	—	—	—	—	—
97-98	—	—	—	—	—	—	—	—	—
99-100	3	—	—	—	—	—	—	—	3
101 & over	—	—	—	—	—	—	—	—	—
Totals	877	154	48	10	1	1	—	1	1092

$$\sigma_{\text{death}} = 7.7560. \quad \sigma_{\text{order}} = .6228. \quad r = .0850 \pm .0185.$$

TABLE VIII.

*Age at marriage and order of birth.*

Years	Order								Totals
	1st	2nd	3rd	4th	5th	6th	7th	8th	
13-14	1	—	—	—	—	—	—	—	1
15-16	2	—	—	—	—	—	—	—	2
17-18	4	1	—	—	—	—	—	—	5
19-20	25	5	1	—	—	—	—	—	31
21-22	116	13	4	1	—	—	—	—	134
23-24	131	28	7	1	1	—	—	—	168
25-26	122	24	7	—	—	—	—	—	153
27-28	116	12	2	—	—	—	—	1	131
29-30	87	16	2	—	1	—	—	—	106
31-32	64	10	9	2	—	—	—	—	85
33-34	55	9	4	—	—	—	—	—	68
35-36	38	7	3	1	—	—	—	—	49
37-38	35	5	3	2	—	—	—	—	45
39-40	19	6	2	—	—	—	—	—	27
41-42	20	1	—	1	—	—	—	—	22
43-44	15	1	1	—	—	—	—	—	17
45-46	4	5	—	1	—	—	—	—	10
47-48	7	1	1	—	—	—	—	—	9
49-50	5	2	2	—	—	—	—	—	9
51-52	4	—	2	—	—	—	—	—	6
53-54	3	—	—	—	—	—	—	—	3
55-56	2	2	—	—	—	—	—	—	4
57-58	1	1	1	—	—	—	—	—	3
59-60	1	1	—	—	—	—	—	—	2
61-62	1	1	—	—	—	—	—	—	2
63-64	2	—	—	—	—	—	—	—	2
65-66	—	—	—	—	—	—	—	—	—
67-68	1	—	—	—	—	—	—	—	1
69-70	—	—	—	—	—	—	—	—	—
71-72	—	—	—	—	—	—	—	—	—
73-74	1	—	—	—	—	—	—	—	1
75-76	—	—	—	—	—	—	—	—	—
77-78	—	—	—	—	—	—	—	—	—
79-80	—	—	—	—	—	—	—	—	—
Totals	882	151	51	9	2	—	—	1	1096

$$\sigma_{\text{age at marriage}} = 3.9069, \quad \sigma_{\text{order}} = .6228, \quad r = .0959 \pm .0182.$$

TABLE IX.

*Age of father at birth of son and order of birth.*

Years	Order								Totals
	1st	2nd	3rd	4th	5th	6th	7th	8th	
15-16	2	—	—	—	—	—	—	—	2
17-18	3	—	—	—	—	—	—	—	3
19-20	2	—	1	—	—	—	—	—	3
21-22	29	3	1	—	—	—	—	—	33
23-24	70	4	—	1	—	—	—	—	75
25-26	82	9	3	1	—	—	—	—	95
27-28	107	8	5	1	—	—	—	—	121
29-30	89	17	3	1	—	—	—	—	110
31-32	95	18	6	—	1	—	—	—	120
33-34	74	15	4	—	—	—	—	—	93
35-36	71	12	2	—	—	—	—	—	85
37-38	60	12	9	—	—	—	—	—	81
39-40	50	13	2	1	1	—	—	—	67
41-42	39	7	5	—	—	—	—	—	51
43-44	23	8	3	—	—	—	—	—	34
45-46	17	8	2	2	—	—	—	—	29
47-48	15	3	—	—	—	—	—	—	18
49-50	10	3	—	1	—	—	—	—	14
51-52	14	1	1	1	—	—	—	—	17
53-54	5	3	—	1	—	—	—	—	9
55-56	4	1	—	—	—	—	—	1	6
57-58	5	1	1	—	—	—	—	—	7
59-60	4	3	—	—	—	—	—	—	7
61-62	1	1	—	—	—	—	—	—	2
63-64	3	—	1	—	—	—	—	—	4
65-66	2	1	—	1	—	—	—	—	4
67-68	—	—	—	—	—	—	—	—	—
69-70	—	1	—	—	—	—	—	—	1
71-72	—	1	—	—	—	—	—	—	1
73-74	—	—	—	—	—	—	—	—	—
75-76	—	—	—	—	—	—	—	—	—
77-78	—	—	—	—	—	—	—	—	—
79-80	—	1	—	—	—	—	—	—	1
Totals	876	154	49	11	2	—	—	1	1093

$$\sigma_{\text{age at birth}} = 4.7216. \quad \sigma_{\text{order}} = .6228. \quad r = .1546 \pm .0171.$$



TABLE X.

*Order of birth and number born.*

		Number born																		Totals	
Year		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		18
Order of birth	1st	126	85	68	83	88	94	94	71	48	39	34	25	19	7	12	2	2	—	1	898
	2nd	21	9	10	21	15	15	14	10	6	12	4	4	6	2	2	—	—	—	—	151
	3rd	8	4	3	5	3	7	2	4	3	6	—	2	—	—	1	—	—	—	—	48
	4th	2	2	—	2	—	—	—	—	1	2	—	1	—	—	—	—	—	—	—	10
	5th	—	—	—	—	—	—	—	—	1	—	—	1	—	—	—	—	—	—	—	2
	6th	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	7th	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	8th	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1
Totals		157	100	81	111	106	116	111	85	59	59	38	33	25	9	15	2	2	—	1	1110

$$\sigma_{\text{no. born}} = 3.5992, \quad \sigma_{\text{order}} = .6228, \quad r = +.0212 \pm .0198.$$

TABLE XI.

	Standard deviation $\sigma_x$	Standard deviation $\sigma_y$	Coefficient of correlation
Age of father at birth of son (1) and number born to son (2)	Birth = 9.44 years	Number = 3.60	$r_{12} = -.020 \pm .020$
Age of father at birth of son (1) and age of son at marriage (3)	Birth = 9.44 ..	Marriage = 7.81 years	$r_{13} = -.040 \pm .020$
Age of father at birth of son (1) and age of son at death or present age (4)	Birth = 9.44 ..	Death = 15.51 ..	$r_{14} = -.062 \pm .019$
Age of father at birth of son (1) and order of birth (5)	Birth = 9.44 ..	Order = .62	$r_{15} = .155 \pm .017$
Number born to son (2) and age of son at marriage (3)	Number = 3.60	Marriage = 7.81 ..	$r_{23} = -.213 \pm .016$
Number born to son (2) and age of son at death or present age (4)	Number = 3.60	Death = 15.51 ..	$r_{24} = .318 \pm .014$
Number born to son (2) and order of male birth (5)	Number = 3.60	Order = .66	$r_{25} = .021 \pm .020$
Age of son at marriage (3) and age of son at death or present age (4)	Marriage = 7.81 ..	Death = 15.51 ..	$r_{34} = .180 \pm .017$
Age of son at marriage (3) and order of male birth (5)	Marriage = 7.81 ..	Order = .66	$r_{35} = .096 \pm .018$
Age of son at death or present age (4) and order of birth (5)	Death = 15.51 ..	Order = .66	$r_{45} = .085 \pm .019$

It is to be noted that of the first four coefficients in Table XI, that is the correlation of age at birth with the other characters,  $r_{12}$ ,  $r_{13}$ ,  $r_{14}$  are small and negative and  $r_{15}$  substantial and positive. The last result is of course expected. Order of birth correlated with the same variables gives small and positive values in every case. Hence it is

obvious that order is a factor, and must be made constant. Whether this arises from biological or social considerations, need not be considered here. The remaining three  $r_{23}$ ,  $r_{24}$ ,  $r_{34}$ , are naturally substantial. We can now proceed to eliminate the disturbing factors, first making the reproductive period constant. We have:

Age of father at birth of son and number born to son. With age at marriage and death of son constant:

$${}_{34}r'_{12} = \cdot 013 \pm \cdot 020.$$

Order of male birth and number born to son. With age at marriage and death of present age constant:

$${}_{34}r'_{25} = -\cdot 037 \pm \cdot 019:$$

Age of father at birth of son and order of male birth. With age at marriage and death of son or present age constant:

$${}_{34}r'_{15} = \cdot 178 \pm \cdot 017.$$

It is to be noted that the partial correlations of age at birth with other variables for constant reproductive period have become positive. Now making order of male birth constant, we have for age of father at birth of son and number born to son:

$${}_{345}r'_{12} = \cdot 082 \pm \cdot 019.$$

The total correlation ratios are as follows:

Mean number born to sons for arrays of father:

$$\eta = \cdot 088 \pm \cdot 020,$$

which if corrected by Pearson's method (*Biom.* VIII, p. 254) becomes indeterminate.

Mean ages of fathers for arrays of number born to sons:

$$\eta = \cdot 194 \pm \cdot 020.$$

If corrected =  $\cdot 11$ .

The regression is apparently not linear. The conclusion however from the above result would appear to be that the later born do tend to enjoy a higher fertility than those born at earlier years, although the intensity of association is not great. As has been already stated, it is probable that age at death as indicating the close of the reproductive period rather overestimates the correction necessary for this event. Accordingly the data were selected in a more stringent way. Only such first born males as survived to the fiftieth year were considered. It was hoped in this case, that as all but a small number of families would be

TABLE XII.

*Age of father at birth of son and number of offspring born to son surviving at least 50 years.*

Years	Number born																			Totals
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
15-16	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	2
17-18	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1
19-20	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1
21-22	4	1	2	1	2	4	2	2	1	1	1	—	—	—	—	—	—	—	—	21
23-24	6	3	3	7	5	8	3	5	2	2	—	2	2	—	1	—	1	—	—	50
25-26	9	2	6	6	3	5	8	8	2	6	8	2	2	1	—	—	—	—	—	68
27-28	10	5	5	11	8	9	10	4	7	4	5	2	4	1	1	—	—	—	—	86
29-30	7	3	4	7	9	7	10	8	3	5	2	2	—	—	1	1	1	—	1	71
31-32	7	6	3	5	10	6	11	5	7	3	2	3	3	—	3	—	—	—	—	74
33-34	6	4	8	—	3	3	7	8	6	7	5	1	—	2	1	—	—	—	—	61
35-36	4	2	1	6	8	5	6	4	3	2	2	3	1	1	1	—	—	—	—	49
37-38	4	—	2	7	5	10	4	4	3	4	2	2	1	—	—	—	—	—	—	48
39-40	5	2	2	1	4	5	5	5	1	1	1	1	—	—	1	—	—	—	—	34
41-42	3	3	1	1	5	3	2	2	4	1	—	1	1	—	—	—	—	—	—	27
43-44	2	2	1	1	1	1	4	4	1	1	—	—	—	—	—	—	—	—	—	18
45-46	—	—	1	1	2	1	2	—	1	—	1	1	1	—	—	—	—	—	—	11
47-48	1	1	—	—	2	1	1	1	—	—	—	1	1	—	—	1	—	—	—	10
49-50	—	2	—	2	—	—	—	—	—	—	1	1	—	—	1	—	—	—	—	7
51-52	1	—	1	—	—	2	—	—	2	—	—	—	1	—	—	—	—	—	—	7
53-54	1	—	—	—	2	—	—	—	—	—	—	—	—	1	—	—	—	—	—	4
55-56	1	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	2
57-58	1	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	3
59-60	—	—	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	2
61-62	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1
63-64	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1
65-66	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1
67-68	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
69-70	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
71-72	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
73-74	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
75-76	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
77-78	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
79-80	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1
Totals	72	36	41	56	69	74	76	64	44	37	31	22	18	6	10	2	2	—	1	661

$$\sigma_{\text{no. born}} = 3.4765. \quad \sigma_{\text{age at birth}} = 3.9348. \quad r = .0367 \pm .0255.$$

completed, any correction for death would be unnecessary and that to make the reproductive epoch constant, we should only have to correct for age at marriage. Unfortunately this restriction rather tends to underestimate the effect of death, as a proportion of the males was reproduced after the limit selected. The coefficients found were:

Age of father at birth of son (1) and number born to son (2):

$$r_{12} = .037 \pm .027.$$

Age of father at birth of son (1) and age of son at marriage (3):

$$r_{13} = .04 \pm .02.$$

Age of son at marriage (3) and number born to son (2):

$$r_{23} = -\cdot214 \pm \cdot016$$

and, making age at marriage constant,  ${}_3r_{12} = \cdot034 \pm \cdot027$ .

The correlation ratios are:

(1) Means of fathers' age for arrays of number born =  $\cdot145$ ; the corrected value is indeterminate.

(2) Means of number born to son for fathers' age =  $\cdot017$ , and the corrected value is also indeterminate.

In so far as in the present case, we are underestimating the effect of age of son at death and in the first series we tended to overestimate it, the true value of the correlation must be between  $\cdot08 \pm \cdot02$  and  $\cdot03 \pm \cdot03$ , a value which though small suggests some real degree of association. Bearing in mind that a negative correlation was to be expected in view of the incompleteness of the record had the true value been zero, it can be inferred that as the age of the father at the birth of the son increases, the family born to the son also increases. A point to be noted is that the later born tend to mate earlier and it is conceivable that there is some association between early marriage and a large family irrespective of the longer reproductive period. It would seem that the average age at marriage during the last century has increased to some extent, which may be simply part of the general change in the country, or because the character of the Peerage has altered. Although heterogeneity in material always causes difficulty in interpreting results, still in the present case it is probable that the values obtained are not seriously prejudiced as the two distributions have the same mode and a general rough similarity.

TABLE XIII.

*Age at marriage of peers of the present and previous generations.*

Age at marriage	Dead	per thousand	Living	per thousand
0—19	5	6	—	—
20—29	294	366	87	302
30—39	338	421	138	479
40—49	127	158	40	139
50—59	29	36	17	59
60—69	5	6	6	21
70—79	3	4	—	—
80 & over	1	1	—	—
Totals	802		288	



The third series of data deals with the possible influences of the age of the grandmother on the number of children born to the mother. The details of this material have been already given in the previous paper and its shortcomings discussed. Unfortunately no correction could be made for age at marriage and present age, though many of the mothers were under forty-five years at the time of inquiry. The material therefore corresponds to our total correlation in the peerage data, with this difference. In the former, the information was obtained through surviving children, in the latter through parents whether living or dead. The fallacies need not be enumerated again. We have

Age of grandmother at birth of mother and number born to mother:

$$r = -\cdot06 \pm \cdot15,$$

which corresponds to the total correlation in the peerage data (Table I).

If the coefficients of correlation found are significant of an actual bias, it will be of interest to see what effect alteration in the reproductive habits of a community are likely to have upon its characters. To solve this problem, we must know for the periods under examination in what way the births have been distributed with respect to the age of the parent at birth. In the following data which were collected through the working of the Education Act, 1907, in Middlesbrough, the age of parent at birth of a school population is given along with the age of the grandmother when the mother was born.

TABLE XIV.

*Age of grandmother at birth of mother and age of mother when child is born.*

	Age of grandmother						Totals
	20 years and under	21st to 25th year	26th to 30th year	31st to 35th year	36th to 40th year	41 years and over	
Age of mother 20 years and under	14	27	30	26	7	10	114
21st to 25th year	59	132	122	98	58	34	503
26th to 30th year	66	144	156	117	74	45	602
31st to 35th year	48	93	103	76	48	7	375
36th to 40th year	27	68	51	47	33	13	239
41 years and over	16	12	21	12	6	7	74
Totals	230	476	483	376	226	116	1907

$$\sigma_{\text{grandparent}} = 1\cdot40. \quad \sigma_{\text{parent}} = 1\cdot23. \quad r = \cdot0013 \pm \cdot012.$$

Mean age grandparent = 29·20 years and parent = 29·42 years.

The standard deviations are:

Age of grandparent  $7.01 \pm .08$  year.

Age of parent  $6.15 \pm .07$  year.

It is evident that these are different distributions.

The mean age of grandparents is  $29.20 \pm .45$  year.

The mean age of parent is  $29.42 \pm .45$  year.

That is, the mean age of parent at birth for the working class population of Middlesbrough in 1901 was .22 year or nearly three months later than in 1871. The difference in comparison with the probable error is not significant, but the standard deviations are significantly different, the older generation being somewhat more variable. On examining the distribution, it is seen that the locus of the means of the curves is not materially shifted, but in the case of the present generation the rise and fall are much steeper.

The following possibilities must be considered:

(1) The extreme units are reproducing faster and hence these mothers are more frequently counted owing to the method of selection. The method of selection was through the children.

(2) The offspring of the extreme births may possess a low survival value, and hence the parents born early and late are not enumerated as frequently as they should be. It is obvious if these causes are operative, as our previous results suggest, they might tend to neutralise each other. Should either be predominant, we should expect some correlation between age of parent at birth of mother and age of mother at birth of child positive in the first instance and either positive or negative in the second, but much smaller. The correlation actually found was  $.0013 \pm .0120$ .

The third and most likely explanation is that in the period under examination the customs of the populations dealt with have changed. The diminution of births at the earlier ages is explained by the alteration in the age at marriage. The following figures taken from the Registrar-General's Annual Report for 1913 give the proportion of married women in the population aged 15-45 years.

Year	Ages			
	15-20 years	20-25 years	25-35 years	35-45 years
1871	1.3	13.9	45.5	39.3
1901	0.7	11.8	46.8	40.7

Hence the fall of births occurring at 25 and under is due to the smaller number of women married. The fall at the end of life finds its most probable reason in the shrinkage in the mean size of the family, dependent on the fall in the birth rate from 102·7 (in 1876) per 1000 women 15–45 to 74·5 in 1901. The actual fall in the population dealt with was not so large as this. If this explanation is correct, we can naturally ask, what effect the alteration in our methods of reproduction will have upon the life expectancy of the succeeding generation? If there be really a negative association between the age of parent at birth of offspring and the length of life of the latter as our previous results indicated, and if the regression were strictly linear, then the tendency should be toward a decrease of longevity. If however the regression be non-linear, as our actual data suggested, those born at maturity living the longest, it might be reasonable to expect a significant increase. We are not in a position definitely to answer this question, but our object will have been achieved if those who are able to carry out a more comprehensive inquiry, make further researches into this rather important subject. The obvious difficulty is to isolate such an effect as we here postulate in view of the undoubted increase in the mean after lifetime at nearly all ages, as shown in the Registrar-General's last life table, much of which can hardly be ascribed to anything but the general improvement of the conditions of life common in greater or less degree to the whole population.

*Age of parent at birth of child and its sex.*

A further series on the same lines as the above was also collected with the object of deciding whether this factor of time played any part in sex determination, and as to whether it offered any explanation of the ratios observed from year to year.

The question however as to whether the sex ratio at birth can be taken as correctly representing the conception ratio, requires investigation before deductions can be drawn from coefficients based on live births. It is probable that the smaller the accident rate, the more likely is the ratio at birth to approach the conception ratio. All observers are agreed that initial births show a slight excess of males (Newcomb, Geissler), and it has been found that a comparatively small number of first births are lost by accident, though a fairly large proportion usually included die during the birth and are still born. Further than this, Cobb using Geissler's data has confirmed the conclusion that

large families show an excess of male births. Now it is a reasonable suggestion that where the family is large, the accident rate must be small, provided we are dealing with a homogeneous population, hence the inference that the alteration in the sex ratio is dependent on a closer approximation to the conception rate, is a feasible one. Turning to the converse aspect, it has been observed that illegitimate children show a preponderance of females in practically all European countries, and it is known that the probability of such living one year is smaller than with legitimate children, especially in the case of males. Hence it is hardly an assumption to say that the illegitimate accident rate is high also. Consequently we should expect a low male-female ratio at birth. In support of this contention, Newcomb from French statistics has shown that the male ratio rises when accidents are included, as well as still births. The following figures are taken from Heape's paper dealing with the population of Cuba. The numbers are large so that the differences may be taken as statistically significant.

	Still births per 1000 births	Sex ratio living births	Legitimate	Illegitimate
White population	31	1084	1076	1040
Black population	57	1012	1067	960

Heape however gives some biological considerations and quotes certain experiments by Vernon (*P. Roy. Soc.* LXV, 1899, p. 350) against the view that the pre-natal death rate is differential, and that a decrease in the accident rate is necessarily followed by an increased male-female ratio.

We find however

	Percentage illegitimate	Marriage per 1000 population	Union by consent
White population	18.72	32.34	7.54
Black population	65.78	9.57	24.57

The number of single men and women is stated to be very large. We can assume from this that promiscuous intercourse must be common and sexual disease may be prevalent, so that beyond the still births, there may have been large numbers of abortions and miscarriages, especially amongst the blacks. Hence the differences of the sex ratios might find a very ready explanation, on the assumption of a heavy pre-natal male death rate. In fact these figures might be used in support of such an argument. Lewis again finds that in rural districts the ratio is higher than in towns. A fact which might be explained as due to the higher accident rate in urban districts.



Rosenfeld (cited by Lewis) gives the sex ratio for still births as varying from 1200 to 1700 and states that their inclusion with live births increases the ratio from 1054 to 1086. Hence the accident rate must have been from 50-220 per 1000 live births. From direct inquiry in Middlesbrough the rate when abortions, miscarriages and still births were included was 70 per 1000.

If we take the male and female death rate at ages for England and Wales from 1838 to 1911 and subtract the one from the other and divide each by the general death rate for each age period, the final result will be indicative of the rate of change in the relative mortality for each group.

The figures are as follows:

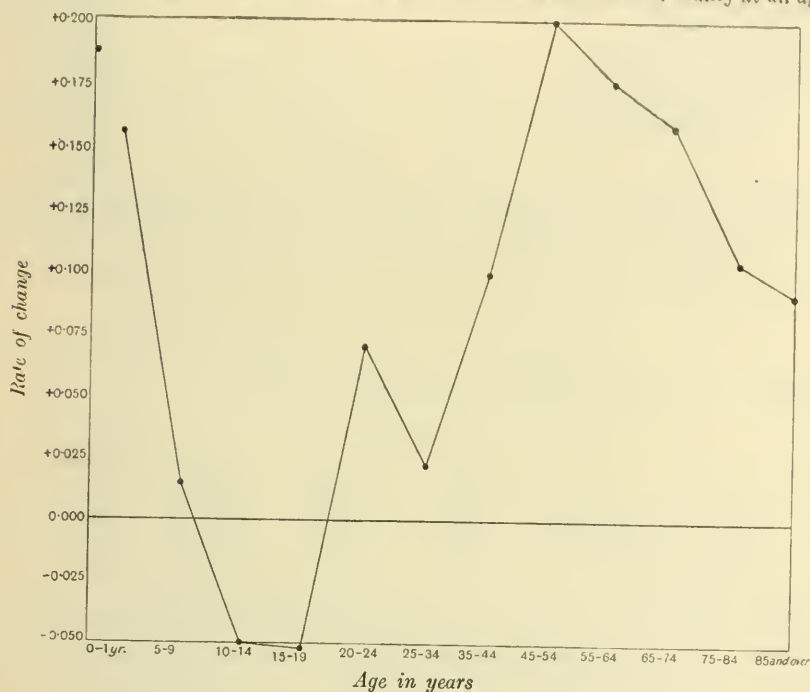
Age period	Male death rate per 1000 living	Female death rate per 1000 living	General death rate per 1000 living	Difference General Rate
0- 1 year	160	132	146	·1917
0- 4 years	65·8	56·2	61	·1573
5- 9 „	6·6	6·5	0·1	·0154
10-14 „	3·7	3·9	3·8	-·0526
15-19 „	5·3	5·6	5·4	-·0558
20-24 „	7·1	6·6	6·8	·0735
25-34 „	8·4	8·2	8·3	·0241
35-44 „	12·2	11·0	11·6	·1034
45-54 „	18·5	15·0	16·7	·2096
55-64 „	33·1	27·7	30·2	·1788
65-74 „	67·8	59·1	63·1	·1378
75-84 „	146·0	131·4	137·7	·1060
85 and over	304·5	277·4	287·8	·0938

The trend of these figures will be appreciated by a glance at the following diagram (p. 147).

It will be seen that from birth to the age group 15-19, the relative disadvantage of the male declines, and consequently the trend of the post-natal mortality figures are consistent with a belief that the male handicap exists, and may even be accentuated, in the ante-natal period. It should be noted that the difference persists for some years after the first and cannot therefore be a mere reflection of the fact that more males die owing to injury at birth consequent upon the larger size of the male head. It has been shown in a previous paper that infantile mortality and pre-natal death rates are highly correlated, and it is hardly an assumption to say that the rates of accidents and miscarriages are even more closely associated with the post-natal death rate during the first month. If therefore we can use this figure as indicative of the pre-natal death rate, for any group of the population, then the deductions

made from the evidence already given would lead us to expect the sex ratio at birth to vary inversely with the death rate in the first month. From the Registrar-General's Report for 1912, the death rates for the

Curve showing rate of change of difference between male and female mortality at all ages.



initial month of life are given for groups according to occupation in the case of legitimate and illegitimate children. Taking the illegitimate according to the occupation of the mother, we have the following figures.

*Group I.* Consists of six subgroups with lowest infantile mortality.

	Number of births		Sex ratio	Death rate 1st month
	Males	Females		
Commercial clerks	127	109	1092	67.8
Milliners	76	51		63.0
Shop-assistants	357	358		46.1
Paper workers	73	70		49.0
Nurses	56	47		48.5
Teachers	57	48		85.7
	746	683		54.6

The sex ratio is high and the occupations consist of selected or higher grade work.

*Group II.* Consists of six groups of highest infantile mortality.

	Number of births		Sex ratio	Death rate 1st month
	Males	Females		
Other workers in dress	53	50	1012	38·8
Wool and worsted manu- facture	268	278		82·4
Barmaids	133	142		69·1
Cotton manufacture	883	824		83·2
Costermongers, etc.	130	141		118·1
Earthenware manufacture	107	120		61·7
	1574	1555		81·8

This group is of a distinctly lower grade than the previous one. In the first case, the sex ratio of 1092 accompanies an initial (first month) death rate 54·6, and in the second case a ratio of 1012 is associated with a death rate of 81·8.

*Group III.* If those subgroups which consist of approximately 2000 births are selected, the same association is observed.

	Number of births		Sex ratio	Death rate 1st month
	Males	Females		
Unoccupied	3751	3622	1035	95·0
Cotton and woolworkers	1151	1102	1044	82·8
Charing and laundry	1371	1441	1051	72·0
Domestic servants	8784	8273	1062	68·5

The association is so marked, that provided the initial assumption is correct, the conclusion must be that the ratio is dependent on the numbers dying before birth. The same point can be illustrated by the legitimate births grouped according to the occupation of the father. The classes are the same as are described on page xli, Registrar-General's Report for 1912.

		Number of births		Sex ratio	Death rate in 1st month
		Males	Females		
Class I	Professional	31,590	30,330	1041	30·2
„ II	Intermediate	56,578	54,504	1038	36·5
		88,168	84,834	1039	34·5
<i>Working Classes.</i>					
Class III	Skilled workman	100,370	96,361	1041	36·8
„ IV	Intermediates	73,396	70,750	1037	38·6
„ V	Unskilled workers	88,517	85,416	1036	42·5
„ VI	Textile workers	12,208	11,920	1024	44·4
„ VII	Miners	50,519	48,692	1038	46·5
„ VIII	Agricultural labourers	16,140	15,634	1032	36·8
Total III-VIII		341,150	328,773	1038	40·4

The association is not so marked as in the case of illegitimate children; this may be due to the grouping or to the special characteristics of some of the classes: thus Class II consists partly of those whose social conditions might place them in Class I or III, and Class VII (miners) is notorious for the low standard of life to which it attains in morals and cleanliness.

In spite of the variations I think we can conclude that considerable support is supplied by these figures to the probable existence of a differential pre-natal death rate.

Turning to the more reliable method of direct observation, the following figures have been collected from numerous sources (Prinzing).

	Males	Females
Living births	106	100
Born dead over 6 months old	130	100
Under 6 months old	160	100

The larger size of the male and its greater difficulty in delivery would account for some at least of the deaths after the sixth month. However we find that out of 3777 still births reported as having occurred in the city of Hamburg in 1903, 2220 or 58·8 per cent. were dead before the onset of labour and 1437 or 38·8 died during delivery; hence the excess of males among still births can hardly be due, in the majority of cases, to mechanical difficulties of delivery. The following figures are quoted from the *Annual Statistical Report of the City of Paris, 1901-2*. Number of abortions out of 100 conceptions:

Age of mother	Percentage of sex		Together	Ratio
	Male	Female		
15-20 years	3·8	2·7	6·5	1·4
21-25 „	3·6	2·4	6·0	1·5
26-30 „	4·0	3·1	7·1	1·3
31-35 „	4·7	3·1	7·8	1·5
36-40 „	5·1	3·2	8·1	1·6
41-45 „	5·2	4·1	9·3	1·3
45 and over	6·5	6·2	12·7	1·1

The ratios for ages over 40, being based upon small absolute numbers, may be neglected and the remaining series does suggest that the male child is more likely to be lost through abortion than the female, and that as the mother increases in age the ratio also increases. For still births (six months and over), according to the age of the mother, the figures are as follows:



Age of mother	Number of still births per 100 born	Males per 100 females
under 17 years	5.9	157
17-20	4.4	132
20-25	4.6	119
25-30	4.8	118
30-40	5.8	123
over 40 years	7.8	123

The excess of males in the earlier years is partly accounted for by the greater difficulties experienced in the initial birth and the large size of the male.

The following figures given by Treichler (quoted from Prinzing) bear this out:

Birth order	Number of children born dead per 100 born
1st	5.1
2nd-3rd	3.8
4th-6th	4.6
7th-9th	5.8
10th-12th	7.9
13th and over	8.4

It will be seen that the rate is highest for first births and we have noted above that there is some excess of males amongst first births. It is also of interest to notice that the death rates, in respect to order, closely agree with similar figures, giving the number dying in the first year after birth. It will be remembered that a high correlation between death before and after birth was found in the previous paper.

The ratio between the pre-natal male and female death rates may be reached by direct or indirect calculation. The direct method is troublesome but is more complete and may be stated as follows. Let  $\alpha$  and  $\beta$  be the numbers of male and female conceptions;  $a_1$ ,  $b_1$ , and  $a_2$ ,  $b_2$  rates of miscarriages and still births; then we have the following conditions<sup>1</sup>:

$$\frac{\alpha a_1}{\beta b_1} = 1.6 \dots\dots\dots (1),$$

$$\alpha a_1 + \beta b_1 = .1 (\alpha + \beta) \dots\dots\dots (2),$$

$$\frac{\alpha a_2 (1 - a_1)}{\beta b_2 (1 - b_1)} = 1.3 \dots\dots\dots (3),$$

$$\frac{\alpha (1 - a_2) (1 - a_1)}{\beta (1 - b_2) (1 - b_1)} = 1.06 \dots\dots\dots (4),$$

$$\alpha a_2 (1 - a_1) + \beta b_2 (1 - b_1) = 0.03 \{ \alpha (1 - a_1) + \beta (1 - b_1) \} \dots\dots (5),$$

<sup>1</sup> Assuming the ratios cited from Prinzing and that 3 % of all births are still births, while 10 % of all conceptions abort or miscarry before viable term.

i.e. five equations between six unknowns, but since the ratio  $\frac{a}{\beta}$  is only required, there are really only five unknowns.

$$\text{Let} \quad A = \frac{a}{\beta},$$

$$\text{then} \quad \frac{a_1}{b_1} A = 1.6,$$

$$A a_1 + b_1 = .1 (A + 1),$$

$$A \frac{a_2}{b_2} \left( \frac{1 - a_1}{1 - b_1} \right) = 1.3,$$

$$A \frac{(1 - a_2)(1 - a_1)}{(1 - b_2)(1 - b_1)} = 1.06.$$

$$A a_2 (1 - a_1) + b_2 (1 - b_1) = .03 \{A (1 - a_1) + (1 - b_1)\}.$$

Solving these equations:

$A$ (ratio of male to female conceptions)	= 1.110,
$a_1$ (male miscarriage rate)	= .1170,
$b_1$ (female miscarriage rate)	= .0811,
$a_2$ (male still birth rate)	= .0329,
$b_2$ (female still birth rate).	= .0270,

and the ratios are:

Male to female live births	1.06,
Male to female still births	1.3,
Male to female abortions	1.6,
Total death rate in first six months	10.0,
Total death rate sixth month to birth	3.0,

which agree with the original conditions.

The series already given with respect to the rising differential death rate after birth can now be completed. The figures run as follows:

Ratio male to female death rate:

First six months	1.44,
Sixth month to birth	1.22,
First year	1.21, etc.

A sequence which confirms the previous deduction.

The following figures taken from Auerbach's paper on the sex ratios in Buda-Pest exemplify the indirect method. The essential difference is that in this case we proceed backwards.

	Males	Females	Death rates		Ratio
			Male	Female	
Born alive	57,142	54,000	—	—	1.057
Born dead	2,030	1,643	.0343	.0299	1.160
Alive before birth	59,172	55,643			
Died at 6th, 7th, 8th month	1,464	1,262	.0241	.0222	1.089
Alive up to 6th month	60,636	56,905			
Died at 5th month	1,340	824	.0217	.0143	1.518
Alive up to 4th month	61,976	57,729			
Died at 4th month	10,583	4,622	.1459	.0741	1.968
Total conceptions	72,559	62,351			

The values differ slightly from those already given, since in the previous calculation only three figures were retained and the calculation was not so detailed, but the order is of course the same.

These results are used in the paper cited to explain the differences found in the sex ratios of certain sections of the inhabitants in Buda-Pest.

The determination of sex in an early embryo is a matter of some difficulty and many must be ignored on the ground that none of the distinctive features are present. In so far as the female genitalia more closely resemble at all stages the primitive state previous to the fourth month, a relatively larger number of males will be ignored than females. Further any abnormality or other conditions which would have led to a state of hypospadias is more likely to resemble a female than a male. The probability is therefore that figures relative to the sex ratio of embryos rather underestimate the proportion of males.

It is of interest to note that Punnett in his investigation on material taken from the census of 1901 and using proportion of servants as an index of nutrition found that if the London boroughs were divided into three groups the following ratios would be obtained:

(1) Less than 15 per cent. indoor servants.	Sex ratio	101.0
(2) Between 15 per cent. and 30 per cent.	„	102.2
(3) Over 30 per cent.	„	103.7
Peerage		107.6

He also points out that infant mortality is high in the poorer districts and that first and second births show some increase in the proportion of males. He concludes that nutrition alone cannot explain the results obtained.

The pre-natal death rate may be however an important factor in determining the sex ratio at birth not only of these groups but also of any community. For example, should the present crusade in this country relative to expectant mothers and the establishment of ante-natal clinics have the desired effect, it would be reasonable to expect as a result of the fall in ante-natal mortality an increase in the male-female ratio.

Unfortunately no data are available in this country with respect to abortions and miscarriages, still material has been collected which may be reasonably supposed to follow closely the ante-natal death rates. To test this hypothesis, as Punnett had already done, the areas constituting the County of London (Census 1911), were selected and for each borough the sex ratio, fertility based on married women 15-45 years, the proportion of servants per 100 families and the mean age of married women 15-45 years were chosen as variables for the following reasons:

I. *Fertility.* This may affect the sex ratio in two ways: (1) first births have a slight tendency to an excess of males (Newcomb), (2) a similar condition has been shown to exist for large families (Cobb, Geissler). It is obvious that the correlation between fertility and sex ratio is non-linear and hence any value of the correlation coefficients obtained will probably underestimate the actual association.

II. *Proportion of Servants per cent.* It is probable that where the amenities and comforts of life are enjoyed to the extent that domestic help allows, the chances of accident before full time and death in the first year will be reduced: the actual accident rate would be infinitely better, but some of the correlation will be shown by using infant mortality as a measure of ante-natal mortality.

III. *Mean age of married women 15-45 years.* This is not synonymous with the mean age at birth of offspring, but it is assumed that the two are highly correlated. A closer approximation to the mean age of reproducing women can be obtained by using Newsholme and Stevenson's method of correcting the birth rate for age distribution. They used the following factors taken from the statistical returns for Sweden:

Age of mother	Birth rate per 1000 married women
15-20 years	518
20-25 „	451
25-30 „	375
30-35 „	312
35-40 „	250
40-45 „	142



The objection to the use of these rates for changing the mean age of married females to mean age of reproducing women is the extreme doubt as to their applicability to the various London areas. It may be observed that the birth rate based on the married women shows little difference from that corrected with the aid of the above age birth rates. The data are as follows:

*Table giving the sex ratio, proportion of servants, fertility and mean age of married women for the London Boroughs.*

Borough	Sex ratio boys per 1000 girls	Fertility per 1000 women 15-45	Number of domestic servants per 1000 families	Mean age of married women
Battersea	·9817	19·80	10·63	33·64
Bermondsey	1·0010	25·26	4·25	33·26
Bethnal Green	1·0430	25·40	3·49	32·95
Camberwell	1·0200	19·74	11·75	33·69
Chelsea	1·0540	17·48	55·07	33·92
Deptford	1·0314	20·92	11·44	33·52
Finsbury	1·0518	24·33	5·02	33·33
Fulham	1·0144	18·97	13·89	33·43
Greenwich	·9659	20·39	18·90	33·78
Hackney	1·0797	19·45	12·86	33·56
Hammersmith	1·0552	18·37	13·50	33·45
Hampstead	·9836	14·13	75·75	34·38
Holborn	·9685	15·71	19·37	33·45
Islington	1·0320	19·33	10·04	33·45
Kensington	1·1075	17·67	72·39	33·96
Lambeth	1·0702	18·90	13·04	33·60
Lewisham	1·0774	16·53	24·64	34·21
Paddington	·9979	17·58	41·85	33·71
Poplar	1·0697	25·12	5·42	33·24
Marylebone	1·0327	17·18	49·35	33·64
St Pancras	1·0670	19·55	12·42	33·22
Shoreditch	1·0443	25·67	3·92	33·19
Southwark	·9891	22·83	5·15	33·14
Stepney	1·0646	25·36	6·43	32·89
Stoke-Newington	1·0691	17·37	20·71	33·87
Wandsworth	1·0093	16·76	24·73	34·07
Westminster	1·0447	13·71	56·44	33·93
Woolwich	1·0522	17·75	12·73	34·01
Means	1·0349	19·688	21·97	33·59
Standard deviations	·0327	3·420	20·64	·33

The coefficients of correlation for the variables are:

Sex ratio (1) and fertility (2):

$$r_{12} = \cdot 091 \pm \cdot 126.$$

Sex ratio (1) and proportion of servants (3):

$$r_{13} = .043 \pm .127.$$

Sex ratio (1) and mean age (4):

$$r_{14} = -.043 \pm .127.$$

Fertility (2) and proportions of servants (3):

$$r_{23} = -.700 \pm .065.$$

Fertility (2) and mean age (4):

$$r_{24} = -.891 \pm .026.$$

Proportion of servants (3) and mean age (4):

$$r_{34} = .762 \pm .054.$$

Making proportions of servants constant for each area we have:

Sex ratio (1) and fertility (2):

$${}_3r_{12} = .169 \pm .124.$$

Sex ratio (1) and mean age (4):

$${}_3r_{14} = -.117 \pm .126.$$

Fertility (2) and mean age (4):

$${}_3r_{24} = -.773 \pm .051.$$

Lastly eliminating fertility we have:

Sex ratio (1) and mean age (4) with proportion of servants (2) and fertility constant (3):

$${}_{23}r_{14} = +.023 \pm .127.$$

Similarly:

Sex ratio (1) and fertility (2) with mean age (4) and proportion of servants (3) constant:

$${}_{34}r_{12} = .12 \pm .13$$

and sex ratio (1) and proportion of servants (3) with mean age (4) and fertility (2) constant:

$${}_{24}r_{13} = .13 \pm .12.$$

The suggestion from these results, were they significant with respect to their probable error, would be that a rise in fertility, number of

servants kept or in mean age of married women leads to an increase in the relative number of boys born.

In so far as a rise in fertility, with other factors constant, may denote a fall in pre-natal mortality and an increase in the proportion of servants under similar conditions may be a factor of an enhanced degree of pre-natal care, the first two results obtained are not inconsistent with the hypothesis that pre-natal mortality is a factor of the sex ratio of any district.

The third result is contrary to what would have been expected, for we have seen that abortions and miscarriages tend to increase with age of parent, and hence if the mean age of married women is indicative of the mean age at reproduction it should be associated with a relative increase of females and the sign of the correlation be negative. Hence the possibility of the nutritive influence of age on the ovum after fertilisation being compensated by an increase in the male-female conception ratios, seems worthy of further inquiry. But in view of the large "probable errors" of the coefficients, no certain conclusions can be drawn from this analysis.

The influence of age of parent at birth of offspring on the number of males and females born alive has been directly investigated by numerous observers from Sadler onwards and nothing very definite has been discovered.

In fact it would almost appear as if the sex ratio at birth, with respect to age of parent, is subject to certain small fluctuations, which differ at different times and in different localities. The following series of observations cannot be compared with the much more numerous series that will be found in the literature quoted. It goes somewhat further however, in so far as the birth order and age of mother have been taken in the same material, whereby partial correlations can be found. The data were obtained through the Notification of Births Act, 1908, in the County Borough of Middlesbrough and the Urban District of Barking Town. All births that occurred in these areas were visited and the necessary information obtained. An important point is the fact that all the still births and a small number of miscarriages are included, exactly what proportion of the latter it is impossible to say. The actual figures are as follows:

TABLE XV.

*Sex and age of mother at birth.*

Age of mother	Number of males born	Number of females born	Total	Sex ratio
15	0	1	1	1.00
16	2	3	5	.60
17	6	5	11	.45
18	19	19	38	.50
19	36	38	74	.51
20	61	58	119	.49
21	68	58	126	.46
22	77	74	151	.49
23	95	96	191	.50
24	84	100	184	.54
25	117	75	192	.39
26	95	92	187	.49
27	71	85	156	.54
28	98	81	179	.45
29	85	85	170	.50
30	101	59	160	.37
31	57	57	114	.50
32	172	81	153	.53
33	63	62	125	.50
34	64	77	141	.55
35	58	54	112	.48
36	46	60	106	.57
37	49	44	93	.47
38	44	48	92	.52
39	44	40	84	.48
40	42	30	72	.42
41	18	16	34	.47
42	24	17	41	.41
43	13	18	31	.58
44	11	11	22	.50
45	8	6	14	.43
46	5	3	8	.38
47	8	1	9	.11
48	2	0	2	.33
49	0	1	1	1.0
	1643	1556	3194	

The coefficients obtained are:

Age of mother at birth of offspring and sex ratio:

$$r = - .055 \pm .011$$

and the mean square contingency:

$$C_2 = .103 \pm .013$$

and if corrected

$$= .017.$$



The suggestion is that as the mothers get older there is a *slight* tendency to produce a larger number of males. Many observers, Newcomb, Geissler and others, have thought that birth order is in some way associated with sex determination. The following figures, taken from the above material, illustrate this point.

TABLE XVI.  
*Order of birth and sex.*

Order	Male	Female	Totals
1st	310	274	584
2nd	210	265	475
3rd	208	184	392
4th	187	174	361
5th	135	126	261
6th	130	116	246
7th	94	95	189
8th	79	71	150
9th	61	53	114
10th	57	44	101
11th	38	34	72
12th	17	20	37
13th	13	8	21
14th	8	7	15
15th	7	9	16
16th	2	3	5
17th	—	—	—
18th	—	2	2
19th	1	1	2
Totals	1557	1486	3043

In this case order of birth and sex ratio:

$$r = .013 \pm .012.$$

The tendency would seem to be for the male excess of first born largely to counteract the male excess for later born. Taking now the correlations already found we have:

Age of mother at birth of offspring (1) and sequence (2):

$$r_{12} = .613.$$

Age of mother at birth of offspring (1) and sex (3):

$$r_{13} = -.055 \pm .011.$$

Sequence (2) and sex (3):

$$r_{23} = -.013 \pm .012.$$

Should sequence be made constant, nutritive influence will be removed to some extent and age of mother at birth of offspring and its sex:

$${}_2r_{13} = -\cdot06 \pm \cdot01.$$

That is, those born during the earlier years tend to be female. Some support is given to this conclusion, by the following figures taken from the 1901-2 and 1912 *Statistical Reports for the City of Paris*.

The six categories from the twentieth year to the fortieth year are very large, hence the ratios are reliable. All still births of more than seven months' gestation are included.

	Males per 1000 females
15 years and under	1000
15-19	1033
20-24	1049
25-29	1058
30-34	1042
35-39	1124
40-44	1016
45-49	930

There is reason to believe that the fall from the fortieth year onwards is due to the increase in the number of male conceptions that abort. Thus, turning to events happening previously to the seventh month, we find the following figures in the above report for the year 1912.

Age of mother	Abortions (embryos to the 7th month of gestation)		
	Male	Female	Totals
15-19	63	48	111
20-24	409	272	681
25-29	389	268	657
30-34	271	157	428
35-39	152	83	235
40-45	35	29	64
Totals	1319	857	2176

From these figures the correlation between age of mother and the sex of abortions was found to be

$$r = -\cdot032 \pm \cdot016.$$

Although the correlation is not large, still the suggestion is that the male rate for abortions increases as the age of the mother increases. This agrees with what happens after birth. Even in view of the fact that this series must necessarily be incomplete, owing to defect in record and the difficulty in determining sex previous to the second month,

it is impossible to explain the previous result regarding the age of mother and the sex of living and still born children, as being due to a differential rate previous to the sixth month of gestation, for the sequence should be the opposite to that actually found. We must now consider age in another way, namely, the effect of the age of the grandparent at the birth of the parent on the relative numbers of the sexes in their families. We have the following data:

Age of grandmother at birth of mother	Number of inquiries	Males per 100 females born
20 and under	229	100 $\pm$ 4.45
21-25	350	103 $\pm$ 3.60
26-30	220	106 $\pm$ 4.54
31-35	198	113 $\pm$ 4.79
35-40	124	102 $\pm$ 6.06
41 and over	73	132 $\pm$ 7.89

Average number of births per mother=6.1.

The data refer to families of which the duration of married life has been at least twelve years. The introduction to the parent from whom the information was obtained was through a child in its tenth year. It will be seen that the proportion of males in the family increases as the age of the grandparent at which the parent was born increases though in one instance only is the difference definitely significant. The probable errors are based on the number of families investigated, not on the gross numbers of individuals.

### *Conclusions.*

I. The imperfections of the data analysed are such that it must be with some considerable hesitation that any decided statement is made concerning the points discussed. Still, some credence can be given to the belief that those born during the declining years of life do enjoy an enhanced fertility, which may however, by the time at which birth occurs, be actually neutralised by the low survival value of their offspring.

II. If we infer from the evidence just presented, that the pre-natal mortality affects males more than females, then since evidence adduced earlier in the paper supports the conclusions, (a) that infant and pre-natal mortality are highly correlated, (b) that infant mortality is higher in the case of elderly parturients and also in the case of parturients themselves the offspring of elderly parents, and (c) that the differential pre-natal rate increases as age increases, it must follow that the

ratio of male to female births should diminish with the age of the parent. But our direct investigation of this point leads, if anywhere, to an opposite conclusion. Hence it must follow that age exerts a direct polarizing influence upon the sexual cell (whether before or after fertilisation cannot even be conjectured) sufficient to neutralise the factors which make for the production of an excess of females.

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## THE STERILISATION OF VACCINES; AND THE INFLUENCE OF THE VARIOUS METHODS EMPLOYED ON THEIR ANTIGENIC PROPERTIES.

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(With one Chart.)

A VACCINE may, in general, be defined as a standardised suspension of killed bacteria. It is true that in a few instances living organisms are employed as vaccines as in the case of the Haffkine prophylactic for Cholera and the sensitised vaccines of Besredka. Notwithstanding these few exceptions it is suspensions of killed bacteria which are in most general use therapeutically as vaccines. According to Wright's method the bacteria employed in the preparation of a vaccine are killed by heat, the temperature actually employed ranging from 53° C. to 65° C. for one half to two hours. This method of sterilising vaccines has been largely employed, but has recently been subjected to much criticism. It has been stated that employment of heat in the sterilisation of vaccines has impaired the value of the vaccine, the heat coagulating albumin, increasing the toxicity and diminishing the immunising properties of the vaccine.

For these reasons other methods have been introduced, especially the addition of antiseptics. Thus Castellani renders his mixed vaccine sterile by the addition of carbolic acid, Dreyer and Ainley Walker employ formalin in the preparation of typhoid vaccine, whilst Nicolle and Blaizot sterilise their gonorrhoeal vaccine "Dmegon" by the addition of sodium fluoride.

In this way it is claimed that vaccines are deprived of all toxic effects and may be administered without provoking any reaction either local or general. It would seem, however, that these effects are claimed chiefly on clinical grounds. By determining the opsonic index in a

series of rabbits inoculated with vaccines sterilised in various ways their antigenic value can be accurately determined and compared.

The organism selected for these experiments was a freshly isolated strain of *M. pyogenes* var. *aureus* (*Staphylococcus pyogenes aureus*). The vaccine was made from 48 hour agar slope cultures at 37° C. emulsified in 4 c.c. of sterile physiological saline solution and were sterilised by heating to different degrees, by the addition of selected antiseptics, and by the application of ultra-violet rays. A single dose of 100 million organisms was given in each case. The rabbits were all large healthy stock animals and blood was obtained in a Wright's Capsule from an ear vein. In every case, the blood used as the control was taken at the same time as the samples from the inoculated animals, and the specimens for counting the number of bacteria ingested by the polymorpho-nuclear leucocytes were made in the usual manner within a short time after the animals were bled. Human leucocytes were employed and the counts were made on 100 cells in each case. All the inoculations of the vaccines were made into the subcutaneous tissues of the back.

The results obtained are set forth in tabular form in Table I and graphically in Chart I.

TABLE I.

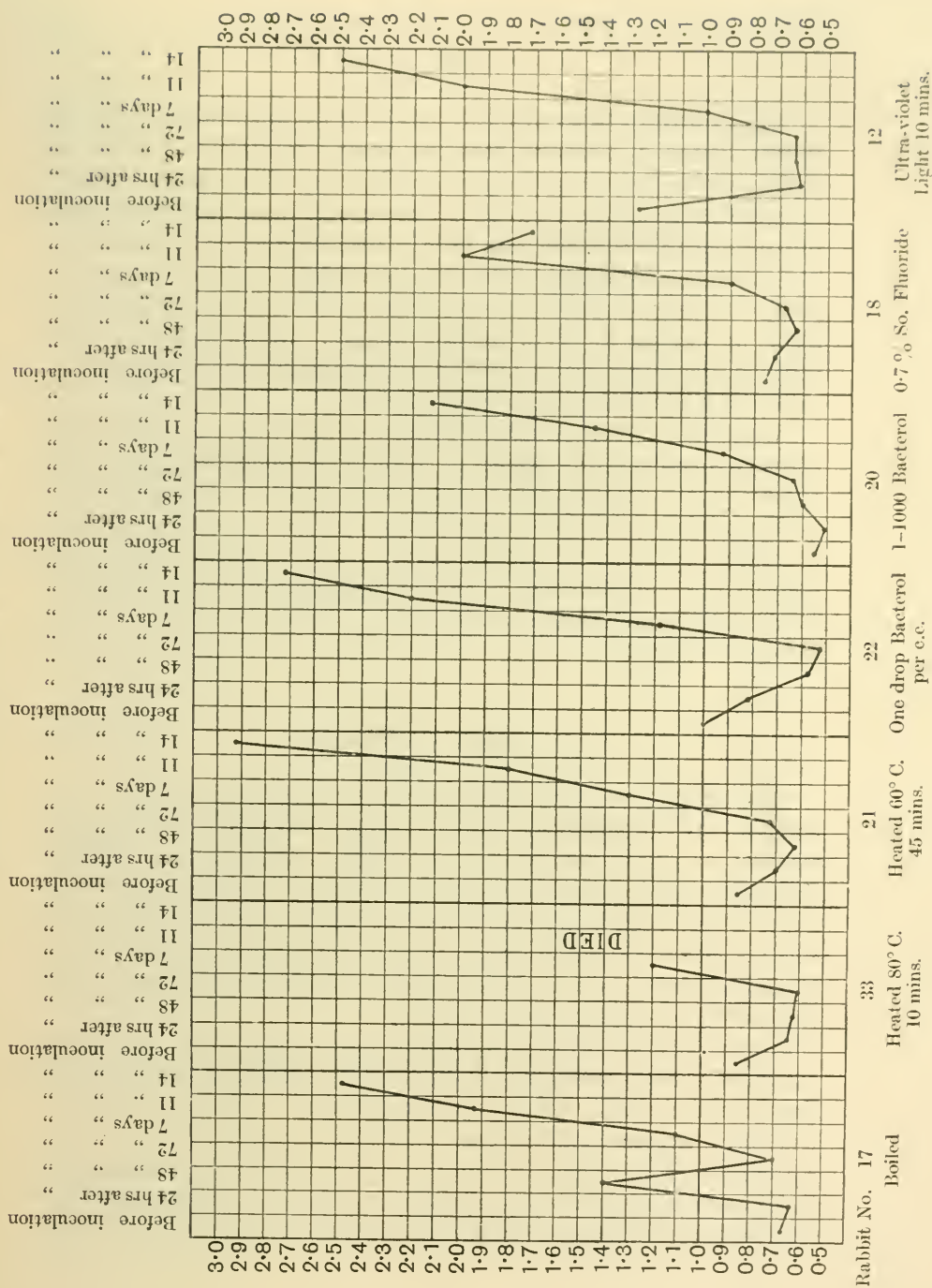
*The opsonic indices obtained by the injection of 100 million staphylococci killed in various ways.*

Rabbit No.	Preparation of Vaccine	Before inoculation	24 hours after inoculation	48 hours after inoculation	72 hours after inoculation	7 days after inoculation	11 days after inoculation	14 days after inoculation
17	Boiled	0.67	0.62	1.40	0.70	1.10	1.93	2.49
33	Heated 80° C. 10 minutes	0.86	0.65	0.62	0.60	1.20	Died	
21	Heated 60° C. 45 minutes	0.86	0.70	0.62	0.73	1.30	1.80	2.92
22	One drop Bacterol per c.c.	1.00	0.82	0.58	0.52	1.19	2.20	2.71
20	Bacterol 1-1000	0.56	0.51	0.60	0.64	0.92	1.45	2.13
18	0.7 per cent. Sodium Fluoride	0.76	0.72	0.63	0.68	0.90	2.00	1.71
12	Ultra-violet Light	1.28	0.61	0.65	0.65	1.00	2.00	2.50

CHART I. Showing the opsonic indices in rabbits injected with a vaccine containing 100 millions of *staphylococci* killed in various ways, as stated.

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*A. Effects of Heat.*

The organism employed was a somewhat resistant strain of *Staphylococcus pyogenes aureus* to heat, as heating to 60° C. needed to be prolonged to 45 minutes to ensure sterilisation. The vaccine caused a negative phase lasting three days, the opsonic index falling from 0.86 to 0.62 and then steadily rose until it reached 2.92 in fourteen days. Thus a pronounced negative phase was followed by a well-marked positive phase. A very similar course was followed where the organisms were killed by heating to 80° C. for ten minutes. At the end of a week, however, the rabbit had a litter of young and some puerperal complication which cut short the determinations.

Where the organisms were killed by boiling there was only a slight negative phase (during which an anomalous temporary rise of the opsonic index occurred) which was followed by a marked rise to 2.49.

Hence the toxicity of staphylococcus vaccines, as determined by a negative phase in the opsonic index, is not increased by raising the temperature, nor does such a rise of temperature exercise any pronounced diminution of immunising power, as determined by the rise in the opsonic index.

*B. Effects of Antiseptics.*

The addition of three drops of pure Bacterol to the three c.c. of emulsion of staphylococci and subsequent shaking in the shaking machine for half an hour ensured the sterility of the vaccine. This is a simple and rapid method of sterilising vaccines. Bacterol is one of the newer disinfectants containing iodine and formalin, which, while possessing a selective action on spores, is possessed of little toxicity so that several c.c. of 3 per cent. Bacterol can be injected intravenously into rabbits without producing toxic effects.

The vaccine so prepared caused a negative phase for three days, the index falling from 1.0 to 0.52, rising afterwards to reach 2.72 on the fourteenth day. Using a 1-1000 solution of Bacterol, three to four days' exposure was necessary to obtain sterility. In this case the index fell in 24 hours from 0.56 to 0.51 and thereafter steadily rose to 2.4 on the fourteenth day. By the use of Sodium Fluoride in 0.7 per cent. solution, as employed by Nicolle and Blaizot, the index fell in three

days from 0.78 to 0.62, then rose to 2.0 on the eleventh day and fell to 1.71 on the fourteenth day.

Hence the substitution of antiseptics for heat does not definitely decrease the toxicity of the vaccine, nor does it increase the immunising power. On the contrary, it does not reach so high a point as in the heated vaccines and in the case of Sodium Fluoride the positive phase is sooner over.

### *C. Effects of Ultra-violet Rays.*

The source of the ultra-violet rays was a quartz mercury vapour lamp using  $3\frac{1}{2}$  ampères, and thin layers of emulsions of organisms were exposed to the rays in a Petri dish with the lid removed, the lamp being placed at a distance of six inches. It was found that the thick suspension of organisms obtained by suspending an agar slope culture in three c.c. of saline solution was not nearly sterilised when exposed in quantities of one c.c. in a Petri dish of three inches diameter. The emulsion of living organisms was standardised by counting according to Wright's method and was diluted down so as to contain 1000 million staphylococci per c.c. On exposure of one c.c. of this diluted suspension it was found that an exposure of five minutes usually sufficed to ensure complete sterilisation. Occasionally, however, a few odd colonies developed on the control cultures and exposure for a further period of five minutes ensured sterilisation. It would thus appear that the ultra-violet rays possess an extremely limited power of penetration of any opaque fluid, and this fact militates against their use in the sterilisation of vaccines.

The vaccine sterilised by exposure to the ultra-violet rays for two periods of five minutes each was used for injection, with the result that the opsonic index showed a very marked fall from 1.28 to 0.61 in 24 hours, and then steadily rose, reaching 2.50 on the fourteenth day.

Hence, in addition to the uncertainty in ensuring sterilisation, ultra-violet rays do not diminish the negative phase or toxicity of vaccines, nor do they increase their positive phase or immunising power.

## CONCLUSIONS.

Taking the opsonic index as a measure of the antigenic powers of a vaccine, its toxicity corresponding with the negative phase, and its immunising powers with the positive phase, it was shown with regard to staphylococcal vaccines that:

(1) Unheated possess no advantages over heated staphylococcal vaccines.

(2) It is unnecessary to limit the amount of heat applied to the minimum that will ensure sterilisation.

(3) Even so high a temperature as boiling does not destroy the antigenic properties of staphylococcal vaccines.

For assistance throughout this work I am indebted to Mr F. Welch of the Bacteriological Department of King's College, on whom much of the labour of making the counts of the opsonic determinations has fallen.

A CONTRIBUTION TO THE EXPERIMENTAL  
STUDY OF FEVER.

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## HISTORICAL.

THE condition of Pyrexia, associated with the febrile state, is a phenomenon which has been noted from the very earliest times, but only since the latter half of the last century has the experimental method been brought in to determine the cause of a phenomenon which had previously been a field of speculation only. The very old ideas can be passed over, but in the last century Johannes Müller regarded fever as a reflex process while Virchow (1853) looked upon fever as a neurosis—the condition being considered analogous to a paralytic state, a failure of the control of the vital functions of the body—while it is interesting to note that Traube (1855, 1864) and Traube and Jochman (1855) at this time determined an increase in the elimination of nitrogen from the body in fever. Both Virchow and Traube assumed the existence of a fever-producing agent, but did not proceed to elucidate its nature or origin.

Panum (1855) extracted from putrifying solutions a heat-stable body, insoluble in alcohol but soluble in water, of which 0.012 grain would kill a small dog, producing febrile disturbances and symptoms of acute infection.

In 1864 Otto Weber and in 1866 Billroth introduced septic material into circulating blood or cellular tissue and they were the first to compare the artificial fever thus induced with ordinary “surgical” fever. They found that the inoculation of putrefactive material, whether of animal or vegetable origin, led to the production of a febrile state reaching its maximum in 2-28 hours. It was also shown that the



products of acute inflammation produced fever under similar conditions and that these products owed their infective properties to the presence of "microphytes." It was concluded that the fever was therefore due to these bodies.

Hermann (1866) obtained results similar to those of Panum and concluded that the body was a protein and worked like a ferment. Burdon Sanderson (1867) prepared from putrid meat a substance, which he called Pyrogen, because, on injection, it produced fever, but it lost its effect on filtration through porcelain. It did not, he says, contain live bacteria, as these were killed by the previous addition of alcohol.

In the same year it was shown by Frese (1866) that the fever-producing properties of the products of decomposition and of inflammatory tissue destruction remain unimpaired after boiling and subsequent filtration.

These results were confirmed by E. Bergmann (1868).

Similarly a febrile reaction has been obtained by different observers after the injection of various specific organisms; by Roussy (1910) with yeasts, by Buchner (1891) with *Pneumobacillus*, *B. pyocyaneus* and *B. prodigiosus*; Klemperer (1892) with *Pneumococcus*, *B. pyocyaneus*, *B. coli* and *B. anthracis*; by Römer (1891) with *B. pyocyaneus* and *Pneumobacillus*, while Hort (1913) investigating emulsions of dead bacteria (vaccines) has found that a fever reaction can fairly constantly be obtained after the injection of *B. typhosus*, *B. coli*, *B. influenzae*, *B. pestis*, *M. melitensis*, *M. catarrhalis*, *V. cholerae*, *Acne bacillus* and certain strains of *Staph. albus*, *S. citreus* and *S. aureus*, while there is a large group of bacteria whose vaccines rarely, if ever, induce fever when injected into rabbits.

Five hours after the injection of .250 gm. of Tubercle bacillus into the normal human being, Koch (1890) noticed shivering and rise in body temperature.

Edelberg (1880), Hildebrandt (1890), Hueppe (1893), Rouques (1893), Krehl (1897) and Zupnik (1903) showed that a febrile reaction followed the injection of various proteins and ferments—though pure enzymes prepared under the direction of Kühne were shown by him to have no thermogenetic property, although commercial enzymes had. Kühne (1893) prepared from Koch's Tuberculin a "deutero-albumose" which, on injection into the body, induced fever, while a fever-producing albumose had also been obtained by Krehl (1897) from the *Bacillus coli communis*, and Matthes (1895) showed that the albumose obtained

from peptic digestion of ordinary proteins produced a similar fever to that produced by the albumose from Tuberculin particularly in tuberculous subjects.

In addition to the above were the common surgical experiences of the fever, which sometimes supervened after more or less severe internal haemorrhage, transfusions of blood, simple fracture, injection of sterile tissue extracts and after severe superficial burns and scalds in which bacterial infection from without could be excluded. In these cases the fever was believed to be due to the liberation in the body of the products of the wholesale breaking down of cells of the body itself.

Following the teaching of Edelberg (1880) and others, the rise in temperature was attributed to the absorption of Fibrin-ferment. Latterly, however, the work of Hort and Penfold (1911-1912) on the effects of injection of ordinary distilled water, in which organisms had propagated to some extent, has shown how careful we must be in accepting the results of the injection of extracts of tissues or other bodies made with such sterilized water. These authors have shown that when ordinary water after it is sterilized is injected intravenously into rabbits (injection dose from 1 in 200 of body weight, *i.e.* 10 c.c. to a rabbit of 2000 gm. weight), the result is usually a short febrile reaction, whereas the injection of pure sterile distilled water, carefully distilled from glass (after the whole apparatus has been thoroughly cleaned out with cleaning fluid—Potassium Bichromate and Sulphuric Acid), produces not a rise, but a fall in the body temperature. When this water is contaminated to varying degrees with some varieties of living or killed bacterial cultures, the fall is, they found, replaced by a plateau and with greater additions by a rise, while with large additions of the same organisms, there is a marked fall in temperature (sometimes preceded by a small rise) ending in death in many cases. They also demonstrated the thermostability of the pyrogen, as well as the fact that it will pass hardware filters, though only to a small extent when packed with gelatin.

These results have been confirmed in the main by other and independent observers. Recently Friedberger (1911) and his pupils have published several papers on the nature of the fever reaction which they regard as of the nature of anaphylactic shock.

Sebastiani (1912) acting along lines previously pursued by Centanni (1894) and Kiliani (1911) has endeavoured to isolate the pyrogen from extracts of bacteria. Using *Bacillus prodigiosus* he takes a 3 day broth culture, and kills the bacilli by heating on the water-bath for 15 minutes,

after faintly acidulating with acetic, hydrochloric or sulphuric acid. He then allows the culture to stand for about 15 days under toluol, precipitates with acetate of lead and removes the excess of lead acetate from the filtrate with 10 %  $\text{Na}_2\text{SO}_4$ . The colourless liquid thus obtained he calls Pyrotoxin. By giving repeated doses of this pyrotoxin at definite intervals and varying doses, he obtained various types of fever—continuous, remittent, intermittent, quotidian—and obtained remission of the fever by lysis or by crisis. He therefore argued that all types of fever were due to one pyrotoxin which acted differently in different cases.

In some of his experiments Sebastiani further purified his pyrotoxin solution by precipitation with alcohol. This removes some substances having a depressant action which may mask the action of the pyrogen itself.

#### *The Author's Investigations.*

In the present investigation *Bacillus coli communis* has been principally employed though occasionally *B. typhosus* has also been used. The animals used in all the experiments recorded were medium sized rabbits. The normal temperature of rabbits has been dealt with by Frothingham and Minot (1912), who showed that it varied irregularly over a span of  $2.5^\circ\text{F}$ . below  $103.5^\circ\text{F}$ .

*Methods used for the Induction of Fever.* At first the method employed by Hort and Penfold (1912) was used, namely, the injection into an ear vein of 10 or 15 c.c. of pure distilled water to which a definite amount of a broth culture of *B. coli communis* or *B. typhosus* was added.

In later experiments, however, the substance used was injected in 1 or 2 c.c. of Ringer's solution and, instead of a broth culture, a preparation of bacteria which could be kept in a dry state was employed.

This was made as follows:

A 24 hour culture of *Bacillus coli communis* on Broth Agar was scraped off with the aid of a little sterile distilled water, and centrifuged—the bacteria not being killed before centrifuging. The bacteria were washed, and some of the bacterial paste left at the bottom of the centrifuge tube was taken and weighed, dried, and weighed again to determine the total amount of dry solids present in a given weight of the paste. The weight of dry solids is taken as representing the amount of dried bacterial bodies present. The remainder of the bacterial paste was then mixed with about an equal weight of anhydrous  $\text{Na}_2\text{SO}_4$ , and was put aside to set. The object of adding the anhydrous



sodium sulphate was to obtain a preparation which would be perfectly dry. Anhydrous sodium sulphate ( $\text{Na}_2\text{SO}_4$ ) in the cold combines with water, forming the hydrated compound,  $\text{Na}_2\text{SO}_4 \cdot \text{Aq}$ . This, when warmed to  $35^\circ \text{C}$ ., gives up its water of crystallisation forming  $\text{Na}_2\text{SO}_4$  in solution, and on cooling again sets to  $\text{Na}_2\text{SO}_4 \cdot 10\text{Aq}$ .

The bacterium-sulphate paste was warmed and cooled several times and was then, when warm, spread out on a plate to cool. When cool it was powdered up in a mortar and the powder thus obtained was used for injection—the dose being usually suspended in 1 c.c. warm Ringer's solution. The powder prepared as above contained 3.8 % of dry bacterial substance. This powder was used in most of the experiments, and six months after its preparation was still potent. In other experiments the pyrotoxin as prepared by Sebastiani (1912) was used.

*Site of injection.* Aseptic methods were used throughout. Generally the injection was intravenous, though similar results were obtained after hypodermic, intramuscular and intraperitoneal injection. The response to the injection was generally sooner after intravenous injection. The fluid injected was always warmed to body temperature before injection.

*Effect of Dosage.* A number of experiments were performed to determine the relationship, if any, existing between the dose of bacterial material injected and the degree of bodily reaction.

Charts 1, 2 and 4 represent such sets of experiments and it is of interest to note the effects (in Chart 1) in the same animal of an injection of the same quantity (15 c.c.) of pure distilled water, containing gradually increasing doses of bacteria. The effects of increasing the amounts of dry bacterial substance injected in a minimum fixed amount of Ringer's solution is well seen in Charts 2 and 4. It will be noticed in all the charts that, with minimum doses, there is produced a rise in temperature which reaches its maximum in 1–2 hours, and then quickly comes back to normal. The effect increases with the dose to a certain point and, on further increasing the dose, the response is a "notched" temperature, *i.e.*, after a single dose the temperature rises, then falls to or below normal and then rises again, sometimes as high as or higher than the first rise, and then falls to normal. With larger doses the temperature may rise very little but the animal is manifestly ill and exhibits the condition described below. On further increasing the dose the temperature falls (sometimes preceded by a slight rise) and the death of the animal occurs in an hour when a sufficiently large dose is injected.



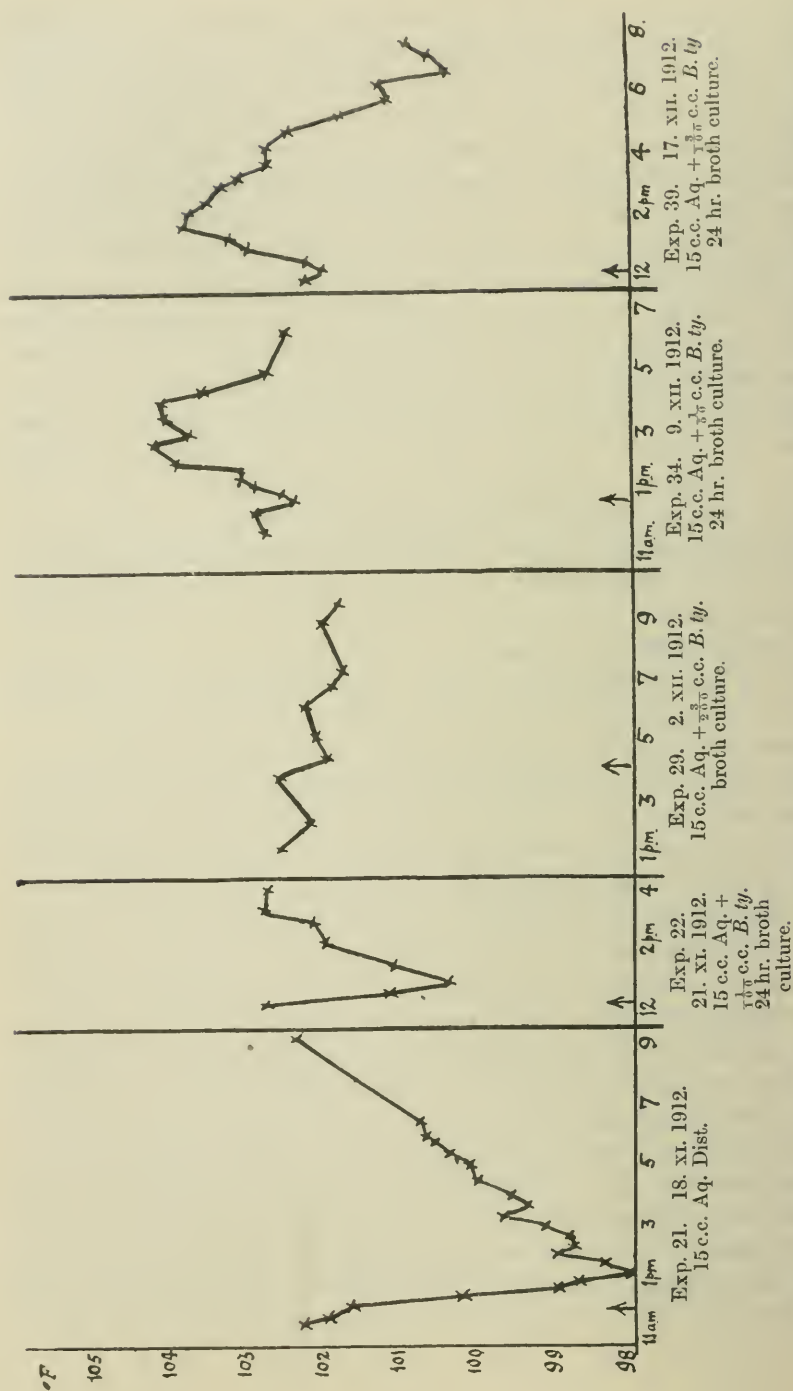


Chart 1. Effect of increasing amount of bacteria suspended in 15 c.c. Aq. Dist. and injected into the same rabbit on successive occasions. Rabbit 18. Experiments 21, 22, 29, 34, 39. (*B. ty.* denotes *Bacillus typhosus*.)

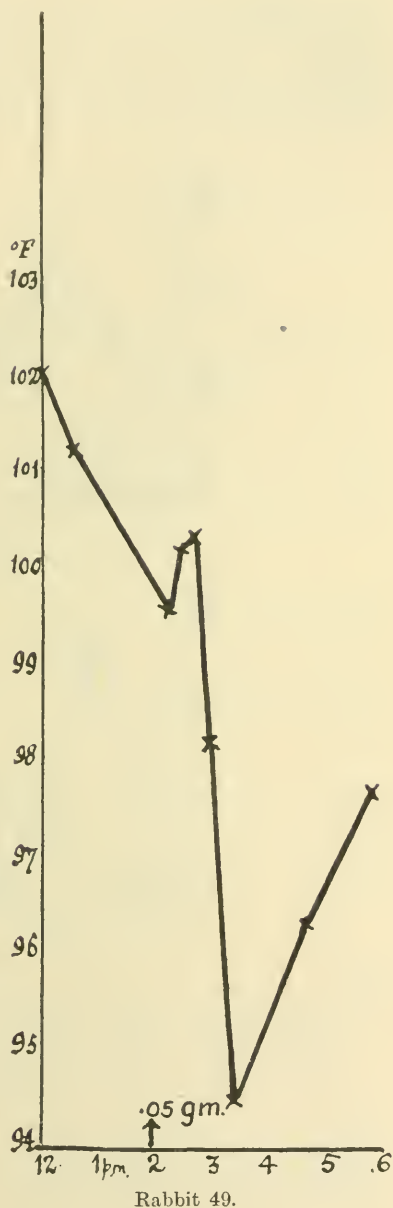
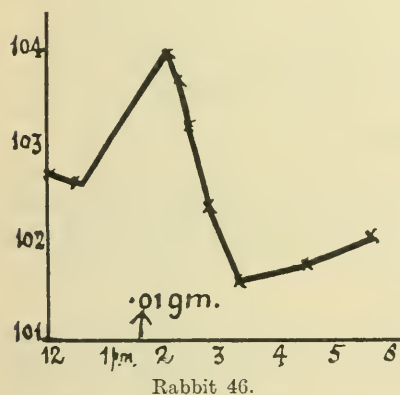
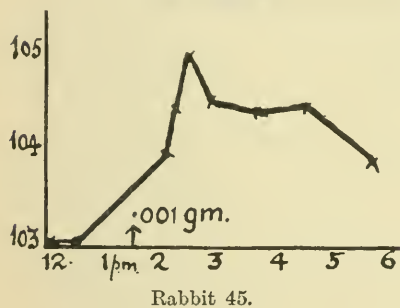
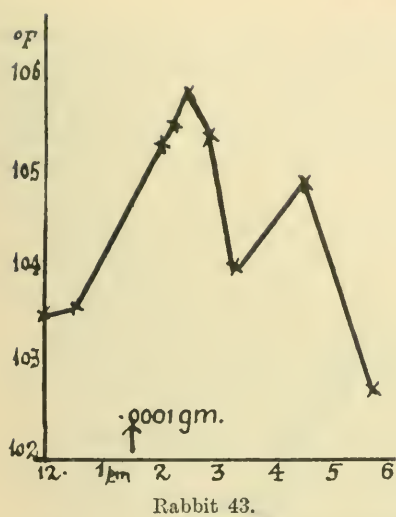


Chart 2. *B. coli communis*=Sulphate powder. Exp. 52. 23. r. 13. Temp. of air dry 13.7° C., wet 11.5° C. Sulphate bacteria powder containing 3.8% dry bacterial substance. Dose in each rabbit injected in 1 c.c. warm Ringer's solution.

*General Effect on Animals of a Single Dose.* The animals reacted to the injection of the pyrogen, either with a temporary pyrexia or a collapse. They showed general signs as well as an effect on the temperature; quietness, lethargy, torpor, sometimes weakness in the legs and even in some cases, inability to stand. More or less marked

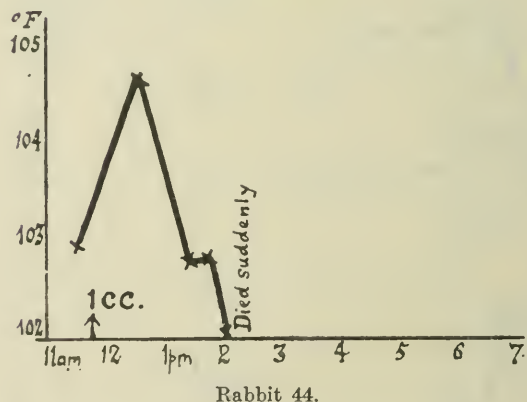
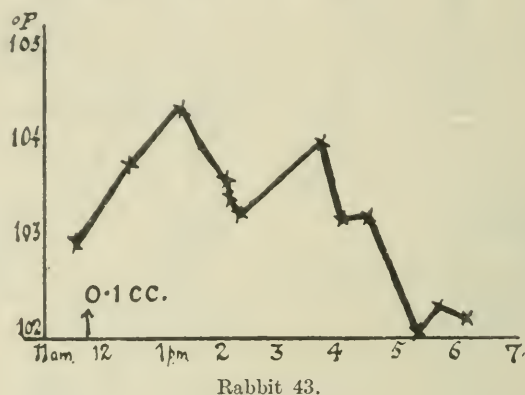


Chart 3. Injection of Bacterium-free supernatant fluid. Exp. 48. 20. I. 13. Supernatant fluid (280 c.c.) from 2.84 gms. dry bacteria; at 2 p.m. No. 44 died suddenly; at 2.10 p.m. No. 43 toppled over and could not stand but regained power in legs in about 3 minutes, at 3.45 p.m. still weak in the legs. Rabbit 43 injected with 0.1 c.c. in 1 c.c. Ringer; rabbit 44 injected with 1 c.c.

Diarrhoea generally followed in an hour or so, and sometimes even the passage of blood by the rectum, which could not be entirely accounted for by the frequent insertions of the thermometer into the rectum.

The exact time relations differed in different animals and with different doses. Generally some effect was noticeable within an hour,

and usually the animal was apparently quite normal again in 6 or 7 hours. In some, however, the effects lasted considerably longer. Types of the different effects are illustrated in the charts.

In one case, Rabbit 44, Experiment 48, Chart 3, the animal died with a temperature of  $102^{\circ}$  F. within  $2\frac{1}{2}$  hours of the injection, the temperature in the meantime having risen to  $104.7^{\circ}$  F.

This animal received an injection of the bacterium-free supernatant fluid obtained by centrifuging a suspension of *Bacillus coli communis* in distilled water.

*Idiosyncrasy of Animal used.* In many cases the dose was repeated after an interval of several days. No marked alteration in the response was given. Friedberger and Mita (1911) say that a previous dose renders the animal sensitive to a later dose, but other observers (Royal Commission on Tuberculosis 1913) have not found this to be the case.

In many cases different animals of approximately equal weight did not give the same febrile reaction with the same dose. One animal giving a simple rise and fall, another a "notched" temperature and a third a temperature approaching the "plateau" type.

It is of interest to note that the fever reaction is best obtained with young adults (rabbits of about 2000 gms. weight). With older (heavier) individuals a dose of pyrogen sufficient to produce a rise in temperature in the smaller animal had no effect, while the larger dose, which in this animal produced a marked fall, generally produces a slight rise in smaller and younger animals.

The Royal Commission on Tuberculosis (1913) found that with normal bovines after the injection of Tuberculin either a rise or fall in temperature occurred, but in the majority of animals no effect was obtained. This result, however, is quite possibly due to the effect of the dosage, variations in which were not investigated to any extent.

In non-tuberculous rabbits with the doses used, no effect was obtained, while in tuberculous rabbits the results varied. Here again the results obtained may have been due to wrong dosage or to an idiosyncrasy on the part of the rabbit.

With monkeys the results were varied, but on p. 79 of the Commission's Report (1913) the temperature of normal monkey 114, chart 42, is seen to drop  $4.7^{\circ}$  F. in 6 hours after an injection, while in chart 44, monkey 98 shows a fall of  $4.47^{\circ}$  F. within the 6 hours after injection. This result the authors regarded as a failure to react.

*The Effect of Multiple Doses* has been investigated by Hort and Penfold (1912) and by Sebastiani (1912), whose results have been



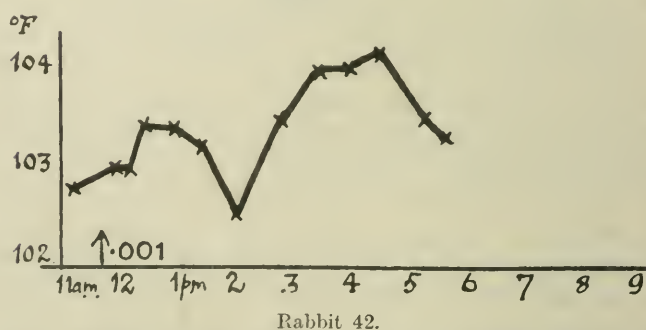
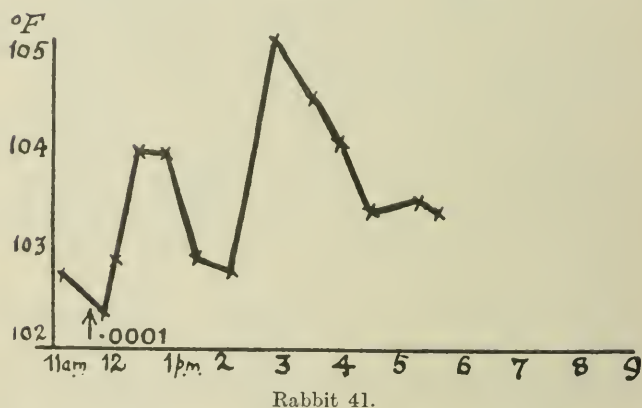
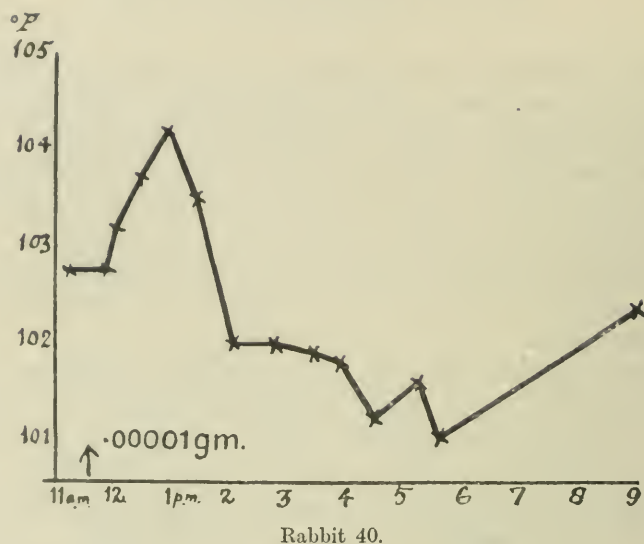
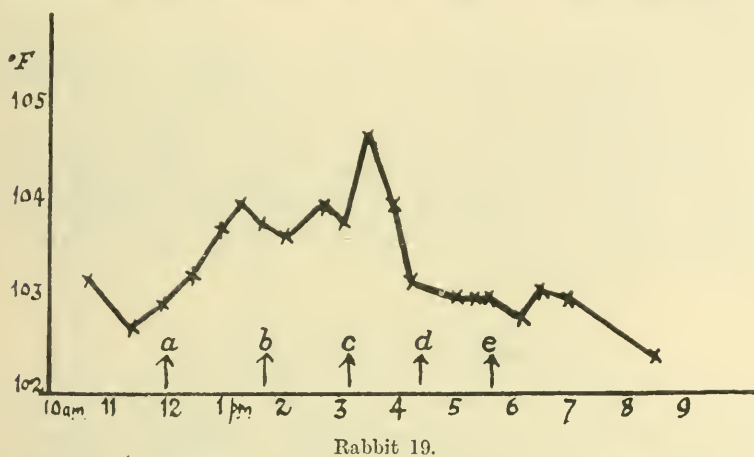


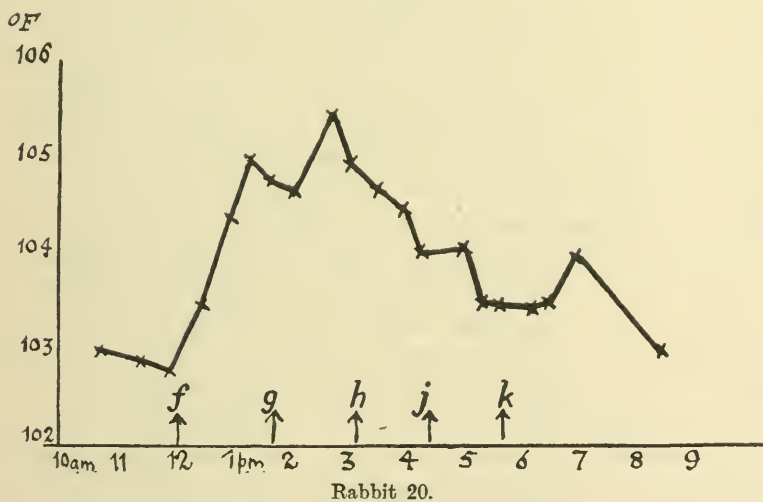
Chart 4. Ether extracted powder. Exp. 55. 27. i. 13. Temp. of air dry 14.4° C., wet 10.0° C. *B.c.c.* sulphate powder as used in Exp. 52 extracted with ether. Rabbit No. 40 injected with 0.00001 gm. *B.c.c.* powder (=0.000004 gm. dry bacteria) in 1 c.c. Ringer. Rabbit No. 41, 0.0001 gm. *B.c.c.* powder (=0.00004 gm. dry bacteria) in 1 c.c. Ringer. Rabbit No. 42, 0.001 gm. *B.c.c.* powder (=0.0004 gm. dry bacteria) in 1 c.c. Ringer (*B.c.c.* denotes *Bacillus coli communis*.)

referred to above. I have also performed a few experiments on this point. Experiment 66, Chart 5, records a result which differs somewhat from Sebastiani's. On giving a series of minimal doses (*i.e.* doses which will produce a small effect though not the maximum) the



Rabbit 19.

Injections: a, 0.00002 gm.; b, 0.00002 gm.; c, 0.00002 gm.; d, 0.00002 gm.;  
e, 0.00005 gm.



Rabbit 20.

Injections: f, 0.0001 gm.; g, 0.00002 gm.; h, 0.00002 gm.; j, 0.00002 gm.;  
k, 0.00005 gm.

Chart 5. Exp. 66. 10. II. 13. Repeated injections of *B. coli communis*—  
sulphate powder.

temperature (Rabbit 19) is seen to rise after each injection by steps to a point which may be regarded as the maximal response and then begins to fall to normal in spite of further injection of a second previously effective dose and of a dose which previous experiment had shown was about the optimum for a "spike" temperature.

A similar effect is seen with Rabbit 20, in which the maximum temperature is soon reached by giving a large initial dose of pyrogen, and whose temperature falls in spite of further injections.

The charts on pp. 107-117 of the Report of the Royal Commission on Tuberculosis (1913) show how the sensitiveness to *Bacillus tuberculosis* diminishes after each injection. A similar result was obtained by Klemperer (1892) with a body obtained from the Pneumococcus which he called Pneumoproteid.

### *Mechanism of the Febrile Reaction.*

It is not proposed to discuss the voluminous literature upon the part taken by variation in heat loss and heat formation in the production of the increased temperature in fever. This has been recently reviewed by O. Loewi in his article "Pharmakologie des Wärmehaushalts" in the *Ergebnisse der Physiologie* (1904), p. 332, by Langlois in the Article "Fièvre" in Richet's *Dictionnaire de Physiologie*, Vol. VI, p. 423 (1904), and by F. Kraus in the chapter "Fever and Infection" in von Noorden's *Metabolism and Practical Medicine*, English translation, 1907, Vol. II, p. 120. A number of observers have however made observations on rabbits in which fever has been produced by the injection of pathogenic micro-organisms which will be briefly summarized. Senator (1873) found intense vaso-constriction of the ear vessels during the rise of temperature following injection of a culture of Swine erysipelas.

May (1893) in a series of extensive calorimetric observations on rabbits found that on the first day the heat loss oscillated round normal, but subsequently augmented to as much as 30 %. W. Rosenthal (1893) by thermo-electric measurements of the temperature of the skin came to the conclusion that in the first stage of the fever there was a constriction of vessels in rabbits. Nebelthau (1895), and Krehl and Matthes (1897) came to similar conclusions from calorimetric observations on rabbits. Most of Nebelthau's experiments lasted over some days. Kalinin (1897), who determined the  $\text{CO}_2$ , N, and  $\text{P}_2\text{O}_5$  output of rabbits in health, and in the febrile condition, also found that at the onset of fever no increase of  $\text{CO}_2$  production occurred.

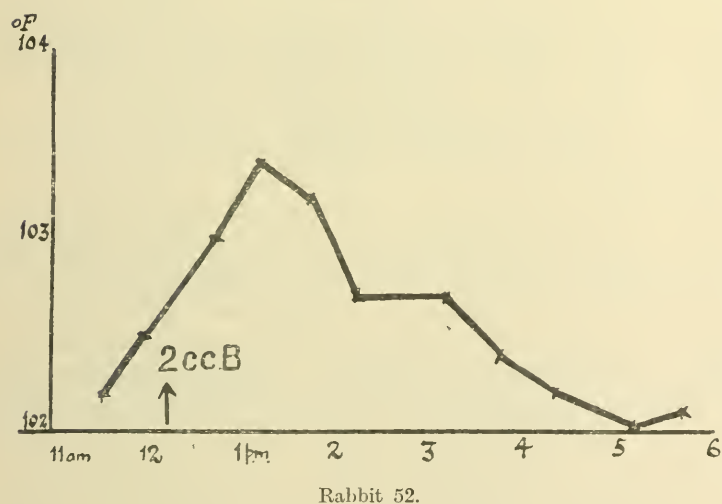
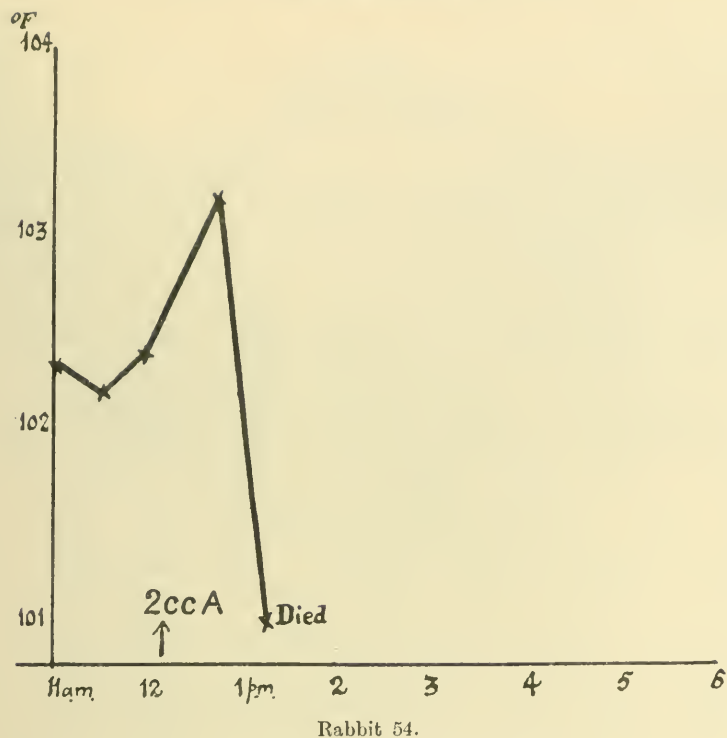


Chart 6. Filtration of pyrogen through collodion. Exp. 83. 1. II. 13. Temp. of air dry  $13.3^{\circ}\text{C}$ ., wet  $10.3^{\circ}\text{C}$ . 24 hour *B. coli communis* broth culture filtered through Berkefeld filter. (A)=filtrate diluted with equal amount of water. 2 c.c. injected into rabbit 54. (B)=(A) filtered through 7% collodion filter at 30 lbs. per sq. in. Filtrate, 2 c.c., injected into rabbit 52.



## A. METHODS.

(1) *Investigation of Heat Loss.*

The reaction of the cutaneous blood-vessels of the rabbit was employed in this investigation as an indication of the amount of heat lost.

The temperature of the ear of the rabbit can be used as an indicator of the condition of the skin vessels if the animal be carefully handled. The large-eared variety, which were used in this particular research, are admirable for this purpose. Their ears present a considerable part of the total body surface which not being heavily insulated as the rest of the body is used by this variety for adjustment of body temperature. The means used was a simple one, and was found sufficiently delicate. Cotton-wool was lightly plugged in the external auditory meatus. The ear was rolled into a tube and kept in this form by two light rubber bands; then a quickly registering thermometer with a very small bulb was inserted into this "ear-tube." (The thermometer would register a temperature of 37° C. from room temperature of 22° C. in 12 seconds.) This thermometer recorded the temperatures of the small air space in the ear, the temperature of which naturally depends on (a) the temperature of the blood in the vessels of the ear; (b) the temperature of the outside air, and (c) the rate of blood flow through the ear. The last is largely dependent on the condition of the blood-vessels of the ear, so that as the external temperature remained nearly constant, changes in the temperature of this air space could be taken as indicating alterations in the vascularity of the ear.

The animal rested comfortably in a small wooden box throughout the experiment.

Experiments showed that, with a fairly uniform rectal temperature, the ear temperature remained fairly constant so long as the animal was comfortable and was not excited or worried, and that its rectal temperature could, by the use of ordinary care and gentleness, be taken with but little reflex disturbance of the skin vessels due to the handling. This can be seen on reference to the Charts 7 and 8 (Experiments 7 and 21 respectively). The animals used in all cases were medium sized rabbits, from about 1600 to 2400 gms. weight (except in one case, Rabbit 50, Experiment 64) and the temperature was always taken at intervals for an hour or more before the injection was given. In many cases the respiration rate was observed, but no observations were made on the actual amount of ventilation.

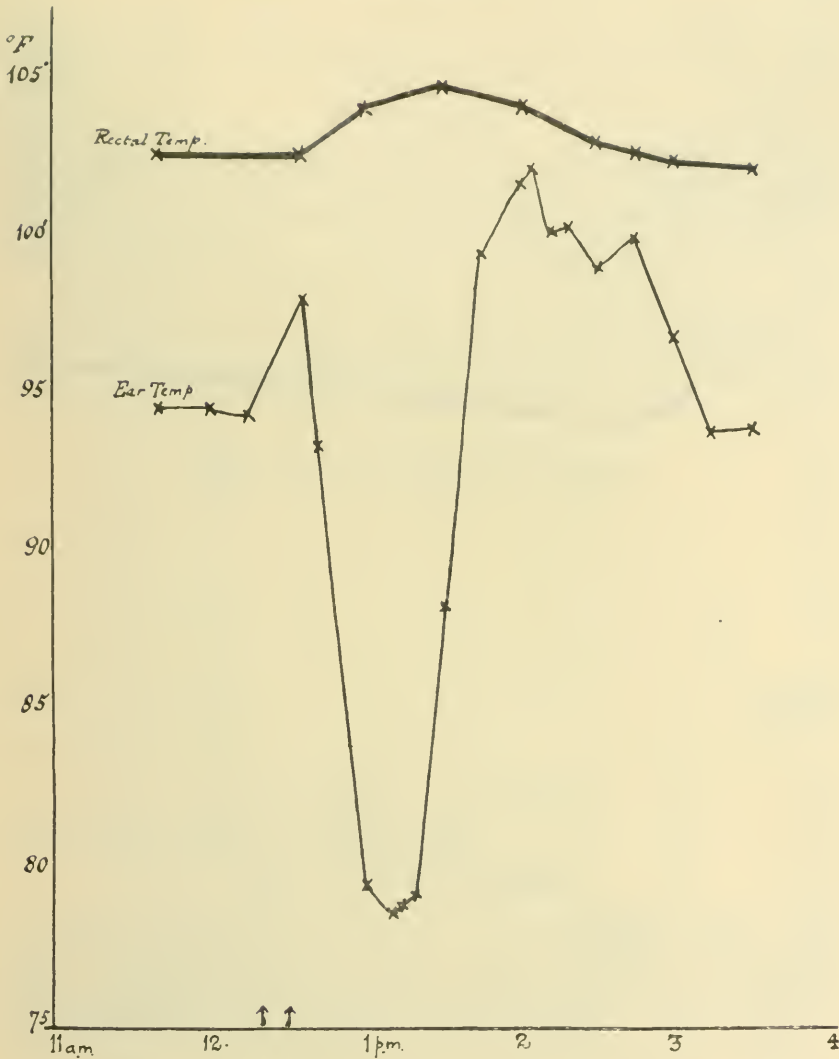


Chart 7. Exp. 7. Temp. of air  $14^{\circ}\text{C}$ ., wet bulb  $11.4^{\circ}\text{C}$ . Rabbit No. 6. Weight before experiment 2820 gms. Loss in weight during exper. (excluding urine and faeces) 100 gms. 12.20 p.m. injected 5 c.c. aq. dist. +  $\frac{1}{200}$  c.c. 24 hr. broth *B. typhosus* culture. 12.30 p.m. injected 5 c.c. aq. dist. +  $\frac{1}{200}$  c.c. 24 hr. broth *B. typhosus* culture.

## (2) Heat Production.

This was determined indirectly by measuring the  $\text{CO}_2$  production and  $\text{H}_2\text{O}$  given off, and calculating the oxygen used. A modification

of the Respiration Apparatus described by Haldane (1892) was employed. Instead of using the large glass bottle which Haldane recommends, a metal box (Fig. 1) was used. Its dimensions were  $40 \times 20 \times 20$  cm., and it had a false bottom of wire gauze, on which the animal rested. Any urine passed while the animal was in the chamber collected in that part of the box below the gauze platform. Inlet and outlet air-tubes were let into opposite ends of the chamber.

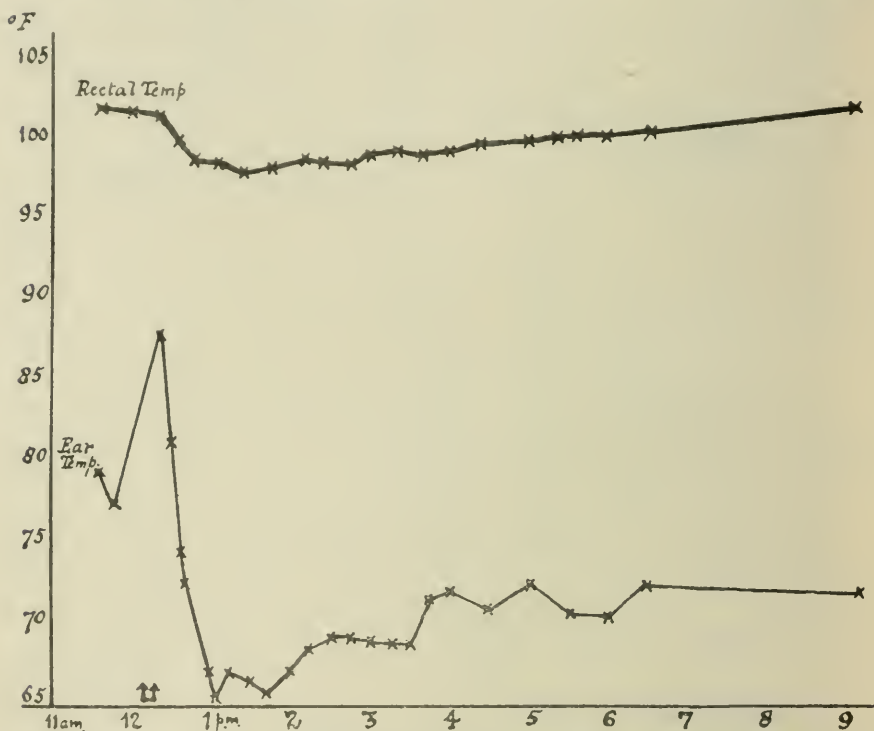


Chart 8. Exp. 21. Rabbit No. 18. Weight before 2252 gms., after 2150 gms., loss in 10 hrs 102 gms. Temp. of air dry  $14.2^{\circ}\text{C}$ ., wet  $11.0^{\circ}\text{C}$  12.10 p.m.–12.18 p.m. injection of 15 c.c. of pure distilled water into ear vein.

The lid was made of thin plate glass, hermetically sealed with red lead into a metal lid the edges of which were flanged so as to fit on the top of the edges of the box. A mixture of vaseline and hard paraffin was used as cementing material. Its weight when completely sealed up was about 4150 gms.

The box was warmed before the animal was put in and the animal was kept in the chamber for half an hour before the experiment was commenced.

## B. RESULTS.

(1) *Heat Loss.*

(1) When an intravenous injection of 10 c.c. distilled water and 1/100 c.c. 24 hour broth culture of *B. typhosus* (boiled) was given (Chart 7, Experiment 7) a rise in body temperature was produced. As the rectal temperature rose, there was a marked constriction of the ear vessels and a marked diminution in the respiratory rate. These reached their minimum before the rectal temperature reached its maximum and then, after a short plateau at this minimum value, the vessels began to dilate and the respiratory rate to increase. The ear

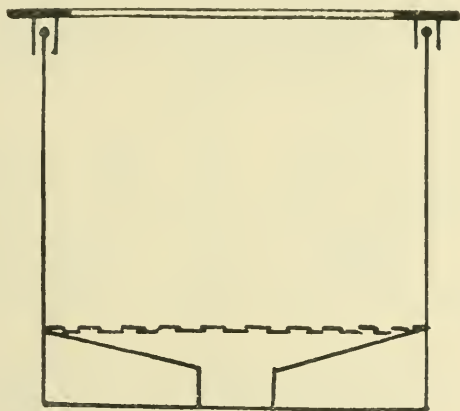


Fig. 1. Transverse Section of Box.

temperature rose above its initial value and this dilatation of the ear vessels was soon followed by a fall in rectal temperature to normal. As the rectal temperature began to fall to its initial level the ear temperature also began to fall to its initial value.

(2) When an intravenous injection of 15 c.c. (warmed) pure distilled water was given, there was a marked fall in rectal temperature (Chart 8, Experiment 21). This was accompanied by a constriction of the ear vessels and a diminution of respiratory rate and these remained at their low value even when the rectal temperature had reached its initial value again. The same effect on the ear vessels is seen in Experiment 64, in which a fall in rectal temperature was produced by the injection of .0011 gm. of *B. coli communis*— $\text{Na}_2\text{SO}_4$  powder containing 3.8% dry bacteria.



*(2) Heat Production.*

A reference to Chart 9 shows that with a rise in temperature induced by injections of suspensions of bacteria (Experiments 80 and 82) there is no consistent alteration in the  $\text{CO}_2$  output. In Experiment 80, 20 % increase occurred during the rise of temperature, in Experiment 82 10 % less  $\text{CO}_2$  was produced. In Experiments 79 and 90 with injections of suspension of fairly large doses of bacteria, there is accompanying the fall in temperature a diminution in the  $\text{CO}_2$  output.

Experiment 87 is a more complicated one, two successive doses of Sebastiani's "Pyrotoxin" being given; and here again there is practically no effect on the  $\text{CO}_2$  output throughout the experiment and so the variations in temperature must be attributed to variations in heat loss. The  $\text{H}_2\text{O}$  output being only partly derived from oxidation in the body and partly from  $\text{H}_2\text{O}$  excreted or evaporated from the lungs or from evaporated urine, no deductions can legitimately be made from the observations recorded.

*Seat of Action of the Pyrogen.* The number of experiments on this point are limited, but on general principles it might be reasonably expected that the pyrogen exerts its action on the body by influencing the central heat regulating mechanism. This is generally regarded as being situated in or in the neighbourhood of the Corpus Striatum (Barbour, 1912).

As far as experiments on the recently "decerebrated" (dethalamate) animal can be of value in determining this point, Experiments 14 and 36 indicate that, after section of the central nervous system distal to the optic thalamus, no fever reaction results from the injection of a dose of pyrogen ordinarily sufficient to induce fever. The operation was performed under anaesthesia. After trephining the skull, an aneurism needle was passed along the tentorium and the brain stem severed below the Optic Thalamus. The brain itself was not mutilated at all.

The position of section was verified by post-mortem examination.

It is, however, questionable whether these results are of much value as the animal was more or less in a condition of shock when the pyrogen was injected.

*Nature of the Fever-Producing Body.* As previously cited experiments have shown, broth cultures of *B. coli* contain pyrogen, though in relatively small quantities. Similarly the washings of emulsions of this organism are also pyrogenetic, although the washings were found to be bacteria free by microscopical examination (Experiment 59b,

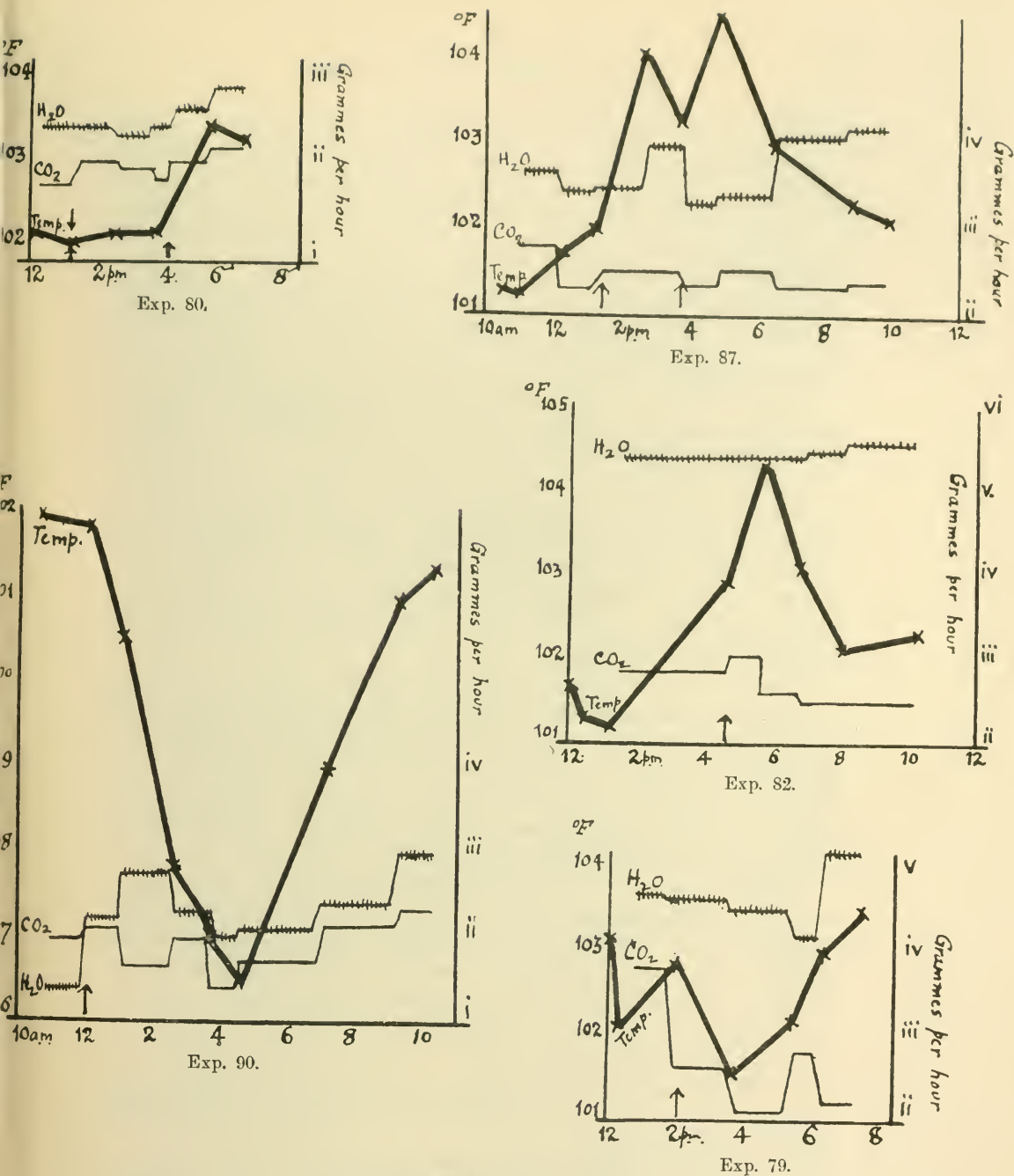


Chart 9. Metabolism in fever.

Exp.	Date	T. of air		Rabbit	Weight	Injection
		Dry	Wet			
79	24. III. 13	13.5° C.	10° C.	59	1600 gms.	0.00018 gm. wet <i>B.c.c.</i> culture (=0.0000176 gm. dry <i>B.c.c.</i> ) in 1 c.c. Ringer
80	26. III. 13	15	11	60	1720 gms.	0.0001 gm. wet <i>B.c.c.</i> culture (=0.00001 gm. dry bacteria) in 1 c.c. Ringer
82	28. III. 13	12.5	10	43	1550 gms.	0.00013 gm. <i>B.c.c.</i> sulphate powder (=0.000005 gm. dry bacteria) in 1 c.c. Ringer
87	7. IV. 13	15.4	10.7	52	1870 gms.	Two separate injections of "pyrotoxin" (Sebastiani) solution
90	9. IV. 13	14.5	10.3	59	1590 gms.	0.05 gm. <i>B.c.c.</i> sulphate powder (0.002 gm. dry bacteria) in 1 c.c. Ringer ( <i>B.c.c.</i> denotes <i>B. coli communis</i> )

Rabbit 41). As previously stated bacterial pyrogen resists boiling. This fact was confirmed by experiment (see Experiment 45, Rabbits 43 and 44). When kept in the form of paste exposed to the air, the pyrogen of *B. typhosus* gradually diminishes in power (Hort and Penfold), but when kept dry in powder form the power of the pyrogen of *B. coli* to induce fever (or in large doses collapse) is, I find, maintained. Drying on the water-bath was found to produce no obvious diminution in potency. It is insoluble in absolute alcohol (see Experiment 70) and its potency is diminished by short contact with absolute alcohol (Experiment 70). It is insoluble in dry ether (see Experiments 53 and 55), but an extract obtained with wet ether produced a moderate pyretic effect (Experiment 46). When a moderately strong solution was filtered through a 7 % collodion filter (made by soaking filter papers in a 7 % solution of collodion in Glacial Acetic Acid, then washing out the acid in running water) at a pressure of 30 lbs. to the square inch, no detectable pyrogen passed the filter (Chart 6, Experiment 83); but when a fairly strong solution was filtered a trace equivalent to about 1/1000 the original amount of pyrogen passed through the filter (Experiments 71 and 74).

Similarly on the addition of colloidal solution of iron to a solution of *B. coli*-pyrogen containing sodium sulphate most of the pyrogen was apparently precipitated with the iron, the filtrate only showing the presence of a trace of pyrogen (Experiment 63). Thus an effect commensurate with that produced by the injection of 0.00001 gm. was obtained from the filtrate from 0.02 gm., indicating that only about 1/2000 of the original pyrogen was left in solution.

With a view to determining the chemical nature of the body responsible for the rise in body temperature, the filtrate in Experiment 71, obtained by filtration of a "pyrogen" solution through a loose collodion filter, and "pyrotoxin" prepared according to Sebastiani, were examined. The results obtained in both cases were:

1. Esbach's Reagent—no precipitate.
2. Biuret Test—negative.
3. Strong  $\text{HNO}_3$ . On floating on strong  $\text{HNO}_3$  a faint green ring at the junction of the liquids was seen and just above the green ring a faint purple ring.
4. Millon's Reagent. On adding the reagent a white precipitate appeared which dissolved in excess of the reagent. The white precipitate also dissolved on warming. No pink colouration occurred.
5. Adamkiewicz (Glyoxylic) Reaction—negative.

6. Phosphotungstic Acid—no precipitate but very faint clouding of the solution.

7. Sulphur test with NaOH and Lead Acetate. No reaction.

8. With  $\text{KNO}_2$  and acid, gas bubbles given off, probably indicating  $\text{NH}_2$  group.

9. On heating, a white substance was left which, on further heating, yielded pungent fumes with an odour like some fatty acid such as butyric. On further heating "ammoniacal" fumes indicating nitrogen, and on further heating, charring indicating carbon.

A sufficient quantity to undertake further chemical analysis has not yet been obtained.

Many of the above tests confirm observations made by Sebastiani (1912).

*Discussion of Results.* From the above cited experiments it will be seen that a substance can be obtained from *B. coli* which, on injection in a minimal amount of Ringer's solution, will induce fever or collapse according to the dose given—a dose equivalent to 0.000004 gm. of bacterial bodies producing fever, and 0.002 gm. producing marked collapse or even death. As the bulk of the weight of the bacteria is something else than pyrogen (compare Sebastiani, 1912), the toxicity of the active substance must be extremely potent.

A comparison of Experiments 48 and 52 shows the relative potency of the bacterial powder and the supernatant liquid in which the bacteria had been suspended, and from which they had been separated by centrifuging. Allowing for idiosyncrasy of the rabbit and taking Rabbit 43 (Experiment 48) and Rabbit 43 (Experiment 52) as giving approximately the same reaction, 0.1 c.c. of the supernatant fluid (Experiment 48) gave an equivalent effect to 0.0001 gm. *B. coli communis* Sulphate Powder (Experiment 52) which contained 0.000004 gm. dry *B. coli communis*.

On the other hand, in Experiment 59, the supernatant fluid used was obtained as follows:

40 c.c. distilled water were added to paste containing 0.72 gm. dry bacteria.

∴ 1 c.c. = Extract of 0.018 gm. dry bacteria (approx. 0.02 gm.).

Again: in the preparation of pyrotoxin according to Sebastiani's method, a suspension of *B. coli communis* was made, and of this 25 c.c. were taken which contained 0.0723 gm. dry bacteria and, in the preparation of the pyrotoxin, this 25 c.c. were diluted to 154 c.c.



This 154 c.c. of "pyrotoxin" solution contained the extract of 0.0723 gm. dry bacteria, or 1 c.c. contained the extract of approximately 0.0005 gm. dry bacteria.

1 c.c. of the pyrotoxin solution (Sebastiani) gave the same pyrogenic effect as 0.00001 gm. *B. coli communis* Sulphate Powder = 0.0000004 gm. dry bacteria.

It is quite probable that the bacterial protoplasm contains many poisons which possess different properties. For example, Sebastiani states that he succeeded in separating a depressing body from the pyrotoxin with alcohol.

Experiment 48, Rabbit 44, and Experiment 83, Rabbit 54, show that there is present in *Bacillus coli communis* a substance of a paralyzing nature for the death of animal 44 in Experiment 48 occurred with a temperature of 102° F., and it is in all probability this body and not the true pyrogen which is responsible for the muscular weakness in the legs often exhibited by the animals. The sudden death of animal 44 in Experiment 48 seems to show that in this case the supernatant fluid took up from the bacteria to a specially large degree a substance having a very severe paralytic action on the body; for one hour after the injection the animal's rectal temperature had risen less than 2° F. and 1½ hours later at the death of the animal had only fallen 1° F. below the animal's temperature at the moment of injection.

The exact chemical nature of the pyrogenetic body has not yet been determined, but from the observations of Centanni (1894), Kiliani (1911) and Sebastiani (1912) it is not protein in nature. It appears, however, colloidal or adherent to bodies in the colloidal state.

It is possible that in the case of burns to the body, substances of a similar nature to bacterial pyrogen are set free in the body and give rise to the fever seen in milder cases and the collapse seen in severe cases. It has been shown by Heyde (1911) that in cases of severe burning there are demonstrable in the urine bodies of a nature similar to the putrefactive amines of the human body. A few experiments in the present research undertaken on this point indicate that when muscle is heated to 200° C. there is a body (or bodies) liberated which exerts an action similar to that produced by injecting a bacterial emulsion. This result was not obtained by subjecting skin to a temperature of 200° C. nor was the effect obtained by injecting muscle which had been heated to 100° C. and 150° C. The sudden fever and collapse seen in cases where an abscess bursts into the peritoneal or pleural cavity is clearly attributable to the rapid absorption of bodies

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contained in the pus or liquor puris or both and which had previously been kept out of the body by the wall of the abscess. The non-septic rise in temperature, which is often seen early in cases of simple fracture with a great deal of effusion, in the first 12 hours of the puerperium, in cases of extensive internal haemorrhage, and after most major operations associated with some degree of tissue destruction, may similarly be due to the entry into the bloodstream of the products of cell disintegration which act in the same way as bacterial pyrogen.

The fact that Ca forms an inert salt with these bodies may account for the so-called antagonism of Ca for pyrogen noted by Hort and Penfold (1912), and may be found of value clinically. So far a few experiments only have been performed and no clear result is yet forthcoming.

That fever-producing substances can be liberated by autolysis of the bodies of bacteria is seen in the method of preparation of Sebastiani's pyrogen (*supra*, p. 171), which has been shown to be much more potent than a simple non-colloidal watery extract of dead bacteria (*vide supra*, pp. 186 and 190).

Undoubtedly some of the results noted above with ferments and with proteoses and other products of protein degradation can be attributed to the association as impurities of simple bodies having a pyrogenic action. In this connection it is particularly interesting to note that Kühne (1893) found that pure enzymes did not exert pyrogenic action.

The results of the skin reaction obtained in the fever studied confirm those observed *in man* by Maragliano (1888, 1890) with the plethysmograph, and those obtained by Geigel *in man* with the thermo-electric method. In the experiments recorded above a different type of fever was examined, all the stages of which were studied in a short space of time (generally about 3 or 4 hours) under the same conditions of atmospheric temperature, humidity and pressure, and uninfluenced by the many considerations, such as diet, attention, etc., which must enter into a study protracted over days.

The result obtained in collapse confirms the generally accepted clinical facts.

With regard to variation in heat production, the results obtained confirm in the main those of other observers, Jaquet (1903), Krehl and Matthes (1897), Loewi (1904), May (1893), von Noorden (1907), and Richter, who state that in the initial stage of pyrexia from infection, heat production is little increased and that the excess of combustion does not exceed 25 %. In collapse with fall of temperature a diminution in heat production has always been observed.

A point of further interest is the pyretic response to alterations in dosage. Here we see in response to increase in dosage of pyrogen beyond a certain point an actual diminution in the pyrexia produced and with a certain dose no pyrexia whatever, while the animal responds to a still larger dose with a subnormal temperature. This kind of phenomenon is met with clinically every day and is at first sight puzzling. In many epidemics of infectious diseases cases often occur showing a high body temperature with perhaps no other signs of intoxication, while other cases are manifestly very ill, yet the temperature is either normal or perhaps subnormal. The response of an old animal with a subnormal temperature to a dose of pyrogen which in a younger animal would give a sharp rise in temperature is met with clinically in the human being in many diseases, *e.g.*, the afebrile pneumonia of old people.

#### CONCLUSIONS.

1. A definite rise in body temperature is obtainable by injecting a minute amount (as small as 0.000004 gm.) of bacterial substance in a small quantity of Ringer's solution and a definite collapse or even death is obtained with larger doses.

2. The body or bodies in the bacteria responsible for the rise in temperature are either of a colloidal nature or adherent to colloids and may be almost entirely separated by a collodion filter. They are soluble in water and saline solutions. They are not destroyed by boiling or by dry heat of 110° C. They are insoluble in ether, and are not affected by prolonged contact with ether. They are insoluble in absolute alcohol, and their potency diminishes after contact with absolute alcohol.

3. The rise in temperature produced lasts a few hours and is principally due to a diminution of heat loss, heat production remaining sometimes unaffected and sometimes increased. The fall in temperature in collapse when large doses of the bacterial extract were administered is due to a diminution in production—heat loss being also diminished.

4. In animals in which the brain stalk has been severed distal to the Optic Thalamus no rise of temperature occurred after the injection of an ordinary pyretic dose.

My best thanks are due to Professor C. J. Martin, F.R.S., for his valuable advice and assistance during the progress of this work.

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# ON THE PATHOLOGY OF BOVINE ACTINOMYCOSIS<sup>1</sup>.

## A PRELIMINARY REPORT.

By FRED. GRIFFITH, M.B.

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### *Introduction.*

ATTENTION has recently been directed to the subject of bovine actinomycosis and its bearing on the public health by the discovery on the part of the health authorities at various ports in this country of large numbers of imported ox tongues showing evidence of actinomycotic infection. A considerable amount of work has been done, both in this country and abroad, upon the pathological condition known as actinomycosis in animals and man, and it has been shown that the disease, in relation to the causal agent, is not a single pathological entity; but in many respects the etiology of the bovine and the human disease and the inter-relationship of the two have not been fully elucidated. With the desire of obtaining fuller information on these matters, the Local Government Board have referred the subject to their Pathological Laboratory for investigation.

<sup>1</sup> Reprinted from *Reports to the Local Government Board on Public Health and Medical Subjects* (n.s. No. 107) 1915, by permission of the Controller of H.M. Stationery Office.—Ed.

This preliminary report deals with the following specimens of bovine actinomycotic lesions which have been sent to the Board's Laboratory for examination between April 8th, 1914, and January 7th, 1915: 50 frozen tongues and lymphatic glands from the lingual region, of which 46 were imported from Argentina, 2 from North America, and 2 from Siberia; 44 fresh specimens from animals slaughtered in this country.

I am greatly indebted to Dr Eastwood for his constant cooperation in this investigation. Acknowledgments are due to the Board of Agriculture and to Professor Woodhead for facilities for certain inoculation experiments; to Dr Littelljohn for organising the collection of pathological material; to Mr Young, Veterinary Inspector for the City of London, Mr Hayhurst, Superintendent, Metropolitan Meat Market, and Mr Cuckney, Brighton Borough Abattoir, for many valuable specimens.

The further stages of this investigation are postponed in view of the emergency work connected with the war.

*Actinomycotic Lesions in Ox Tongues Imported from Foreign Countries.*

As evidence of the frequency of the disease, I may mention that Dr MacFadden, in his report on the work of Inspectors of Foods during the year 1913-14, states that between August 1st and October 28th, 85,445 tongues were examined in the City and Port of London, of which 4949, or 5·8 per cent., were found to be affected with actinomycosis, chiefly in the glands.

With one exception, the forty-six Argentine specimens received at the Laboratory for examination consisted of diseased lymphatic glands which had been removed from forty-five tongues; the remaining specimen was a diseased tongue. The lesions in these glands presented the characters of actinomycosis, and, though individual cases differed in certain macroscopical features, could by summary microscopical examination readily be identified as of similar nature. Occasionally tuberculous lesions were found in lymphatic glands affected also by actinomycosis.

Of the total number of Argentine specimens received and identified as being of an actinomycotic nature, twenty-six were subjected to a more minute examination, which included the isolation of the specific granules under the microscope, the application of various staining

methods, particularly Gram's, and the attempt to recover the causal organism in culture. A histological examination by means of paraffin sections was made of eleven cases.

The following is a summary of the anatomical and microscopical characters of the lesions in the lymphatic glands. In some glands there were single nodules not larger than a small pea, while in others the whole glandular tissue was replaced and there was a considerable increase over the normal size of the gland. The smallest lesions were grey, the largest yellow or reddish. They all consisted of soft moist granulation tissue, occasionally semi-purulent, surrounded by a capsule of fibrous tissue. The abundant formation of fibrous tissue was a characteristic feature, and the central soft tissue appeared to be under pressure, as it usually projected beyond the cut surfaces when a nodule was incised. In the granulation tissue, just visible to the naked eye like minute grains of sand, were numerous orange or yellowish foci. They were fewest in numbers in the early grey lesions and very numerous in the more advanced, to which they gave the characteristic yellow or orange pigmentation. They were generally gritty, and upon the presence or absence of calcareous matter appeared to depend the variation in colour, the soft, non-gritty granules being translucent and of an amber tint. Microscopically these foci or granules were composed of masses of radiating clubs, which either met at a point towards the centre or surrounded a central finely granular area. The clubs did not show a characteristic positive reaction when stained by Gram's method, though they sometimes offered a considerable resistance, most marked in the older lesions, to decolourisation by alcohol, retaining partially and irregularly the violet colouration. The centre of a granule never showed a Gram-staining mycelium, but was usually of a finely granular nature, and therein small aggregations of a minute bacillus could be demonstrated when stained by carbol-fuchsin or methyl violet. The clubs could be well stained in sections by the following methods: (1) carbol-fuchsin, decolourisation by weak hydrochloric acid, counter-staining with methylene blue; (2) Van Gieson's stain; (3) aniline gentian violet, followed by rapid treatment with alcohol.

Numerous attempts have been made to obtain the causal organism in culture from these lymphatic gland lesions, but without definite success. It is possible that the organism had died out on account of the time which had elapsed since the animal was killed, during which the tissues were maintained at a low temperature. Against this assumption, however, there may be mentioned the occurrence in culture

from two of the Argentine cases of an organism morphologically similar to one which has been obtained from fresh cases in this country. It was not grown in pure culture.

Inoculation experiments with emulsions of tissue from the actinomycotic glands into guinea-pigs (subcutaneously and intraperitoneally), a pig (subcutaneously), and a monkey (subcutaneously) have not been successful in the production of lesions.

The following is a description of the single Argentine case examined, in which the tongue itself was infected. Beneath the epithelium of the posterior portion of the dorsum of the tongue were scattered nodules, up to a centimetre in diameter, consisting of fibrous capsules enclosing yellowish gritty tissue which was softening and sometimes semi-purulent. The lymphatic glands in this case were not affected. Histological sections showed granulation tissue containing small masses of radiating clubs which were Gram negative, and surrounded a central granular area staining with eosin. Cultures were unsuccessful.

Two tongues from North America were examined. They showed fibrous nodules beneath the epithelium of the posterior half of the dorsum and sides of the tongue. The nodules were numerous and widely distributed, and ranged up to a pea in size. On section they contained soft granulation tissue beset with minute granules, some yellowish and just visible to the naked eye. On microscopical examination the granules were not gritty, but were of a gummy consistency, translucent, irregular in shape, soft when moist but hard and brittle when allowed to dry. Histological sections showed that the granules consisted of masses of radiating clubs surrounding a finely granular central area, in which no Gram-staining organisms were present. Cultures remained sterile.

From Siberia two frozen tongues were examined. The blades were not affected, but a lymphatic gland at the root in each case contained small nodules of soft yellowish tissue surrounded by fibrous capsules. Histological sections showed the usual masses of radiating clubs and absence of central Gram-staining mycelium.

The disease in the North American and Siberian material appeared to be of the same type as in the Argentine material.

Important contributions to the subject of actinomycosis have been made by Lignières and Spitz. In an article entitled "*L'Actinobacillose*<sup>1</sup>," these authors described an organism which they proved to be the cause of various forms of disease in cattle clinically identical with

<sup>1</sup> *L'Actinobacillose. Bull. Soc. Centr. de Méd. Vétér.* 1902.



actinomycosis, and to which they gave the name of actinobacillus. Their results were unreservedly accepted by Nocard. At the period at which they made the discovery, the disease occurred in the Argentine in epizootic form, attacking 50 to 70 per cent. of the members of a herd. In a second publication<sup>1</sup> they discuss the question of the classification of affections known under the name of actinomycosis, pointing out the impossibility of accepting actinomycosis as a single pathological entity. They describe three distinct groups of actinophytoses, the existence of which their own work has enabled them to establish—(1) *Actinomycosis bovis* (Harz) or *Actinophytosis à Streptothrix actinomyces*, (2) *Streptothrix israeli* or *Actinophytosis à Streptothrix israeli*, (3) *Actinobacillosis of Lignières and Spitz* or *Actinophytosis à Actinobacille*. The second name in each case is in accordance with a method of classification suggested by Lignières and Spitz, to which it is impossible to do justice in a brief summary.

*Actinomycotic Lesions in Cattle from the British Isles.*

Specimens of actinomycosis have been obtained from oxen slaughtered in this country in forty-four instances. It is possible to divide these cases into two groups by the application of Gram's method of staining either to smear preparations of the actinomycotic granules or to histological sections of the pathological tissue. In the first group the clubs are the more prominent feature, and no Gram positive organisms are demonstrable; the second group is characterised by the presence of Gram-staining organisms.

GROUP I.

*Summary of anatomical and histological characters.*

Of the above cases, forty fall in this group. The lesions were found in one or more of the following situations: cheek, floor of mouth, palate, tongue, posterior pharyngeal glands, submaxillary glands, and glands in relation to the tongue. The anatomical and histological features of the lesions were identical with those found associated with the imported tongues. To recapitulate briefly, the lymphatic glands contained circumscribed areas of soft granulomatous tissue, sometimes replacing the whole of the gland. These areas were surrounded by fibrous tissue, and the central portions often contained thick yellowish

<sup>1</sup> Contribution à l'étude, à la classification, et à la nomenclature des affections connues sous le nom d'actinomycose. *Centralbl. Bakt. etc. Abt. Originale*, xxxv. No. 4.

pus. In the pus or the granulation tissue were large numbers of the characteristic granules. The tongues contained varying numbers of nodules similar in structure to those in the glands, situated, in early cases, just beneath the epithelium, and, in the more advanced, extending deeply into the muscle and surrounded by dense fibrous tissue. The lesions in the other situations were similar. Microscopically the specific granules in the tongue nodules, as in the glands and elsewhere, were composed of masses of radiating clubs. By Gram's method the clubs retained irregularly the violet colouration, but no filaments or organisms staining by Gram were present. In the sections stained by carbol-fuchsin or methyl violet occasionally small patches of a minute bacillus could be demonstrated between the clubs or in the central portion of the granule.

*Details of cases investigated.*

The following is a brief description of the individual lesions investigated in the above forty cases:

1. One posterior pharyngeal gland contained four nodules up to .5 or .6 cm. in diameter, composed of a yellowish granulation tissue in which were imbedded minute yellowish gritty foci. Cultures positive.
2. A gland at the root of the tongue on one side contained several small lesions up to .4 cm. in diameter, the smallest grey, the larger yellow and showing minute gritty granules. The tongue was not affected. Cultures positive.
3. A gland at the root of the tongue on one side contained a nodule 1 cm. in diameter. This was composed of soft greyish tissue surrounded by a fibrous capsule and subdivided by fibrous strands. In the soft tissue were small yellowish gritty granules. The tongue was not affected. Numerous organisms in culture not identified.
4. The tongue showed just below the epithelium of the dorsum a number of small discrete fibrous nodules up to a pea in size. On section these nodules contained a softer grey tissue in which were imbedded minute irregular yellowish granules not obviously gritty. The lymphatic gland at the root on the same side contained similar grey nodules. Cultures remained sterile.
5. A lymphatic gland at the root of the tongue on one side contained a lesion about 1 cm. in diameter, composed of soft grey tissue with yellowish granules and surrounded by a fibrous tissue capsule. The tongue was not affected. Cultures positive.

6. A lymphatic gland at the root of the tongue on one side, roughly spherical and about 5 cm. in diameter, showed on section a nodule about 3 cm. in diameter. This was composed of a soft translucent tissue which projected beyond the cut surfaces and was contained in a capsule of fibrous tissue. The gland showed smaller areas similar to the above, and where they approached the capsule there were some fibrous adhesions. The characteristic minute granules were found in all. Cultures positive.

7. The head of an ox was examined. The right posterior pharyngeal gland, measuring  $7 \times 5 \times 3.5$  cm., was hard, and on section more than half replaced by two nodules which were similar to that described in No. 6. At the angle of the jaw on the same side was a gland  $6 \times 5.5 \times 3$  cm., almost entirely replaced by similar tissue, the central portion of which had softened and become purulent. The left posterior pharyngeal gland contained a single small nodule in the centre. The tongue and jaw were not apparently affected. Cultures positive.

8, 9. Lymphatic glands at the roots of tongues contained small lesions similar to those described above. Cultures not attempted.

10. The roof of the mouth showed a hard fibrous tumour, and the upper lip was thickened and hard. On section the fibrous tissue was beset with small areas of soft granulation tissue containing minute granules. Numerous organisms in culture not identified.

11. The posterior half of the tongue was infiltrated by numerous sub-epithelial nodules ranging up to 2 cm. in diameter. On section they presented the appearance of soft granulomata surrounded by fibrous tissue and beset with minute granules. A few of the larger nodules were purulent in the centre. Cultures positive.

12. The tongue was affected similarly to the above, but was in a more advanced stage. Several of the nodules projected through the epithelium on the dorsum, forming flat warty elevations, the largest  $7.5 \times 4.5$  cm. The nodules extended also deeply into the muscles of the tongue. A lymphatic gland on one side contained a nodule about 1 cm. in diameter. Minute granules were present in the lesions in both situations. They were very numerous, and the majority were markedly calcareous. Cultures positive.

13. The tongue was affected similarly to No. 12, but slightly less extensively. No lymphatic glands were involved. Cultures negative.

14. The tongue was infiltrated in the posterior half with nodules beneath the epithelium. Granules were numerous, of a gummy consistency and not gritty. Cultures positive.

15. A lymphatic gland at the root of the tongue contained small soft yellow lesions. Cultures not attempted.

16. On the inner side of the cheek of a bullock were a number of nodules up to 1 cm. in diameter, consisting of fibrous tissue enclosing soft yellow substance in which were numerous minute granules. Several of the nodules had broken through, forming fungating ulcers on the buccal surface. Cultures negative.

17. The tongue showed several sub-epithelial nodules similar to those described above. Cultures positive.

18-22. In each case the lymphatic glands at the roots of tongues showed granulomatous areas with the characters above described. Cultures from two were positive; from three they were not attempted.

23<sup>1</sup>. The tongue showed beneath the epithelium of the posterior half a number of small fibrous nodules up to a pea in size, a few extending into the muscle. The lymphatic glands at the root on each side contained soft grey nodules with yellowish granules surrounded by fibrous tissue and subdivided by fibrous strands. Cultures positive.

24. Beneath the epithelium of the hard palate were small grey fibroid nodules presenting the appearance described above, and containing yellowish gritty granules. The tongue was extensively infiltrated by similar nodules. The material was formalised. Cultures not attempted.

25<sup>1</sup>. Beneath the epithelium of the tongue and extending deeply into the muscle were a number of nodules ranging up to a hemp-seed in size. Cultures negative.

26. In the posterior half of the dorsum and sides of the tongue were about fifteen hard sub-epithelial nodules, slightly prominent, the largest extending into the muscle and measuring up to 1 cm. in diameter. They were composed of fibrous tissue with a grey or yellowish granulomatous centre containing numerous granules. A small lymphatic gland on one side contained a yellowish nodule full of gritty granules. Cultures positive.

27. A portion of the posterior part of the tongue was hard, and infiltrated with fibrous tissue and small grey nodules extending deeply into the muscle. Cultures negative.

28. Tongue showed some superficial ulceration of the epithelium. Immediately beneath and in the muscle were fibrous streaks and very small nodules. Cultures positive.

<sup>1</sup> This case and case 25 were obtained from Irish cattle.



29. Cheek of ox thickened, and on section infiltrated with fibrous tissue and small hard nodules. The nodules were close beneath the epithelium of the mouth, and there was ulceration at one point where the teeth came in contact. Cultures positive.

30. Pharyngeal gland containing a yellowish nodule which projected above the cut surfaces of the gland and measured 1.7 cm. Cultures sterile, excepting a few moulds and colonies of cocci.

31. Submaxillary gland containing a collection of small grey nodules separated by fibrous tissue. Numerous granules consisting of masses of clubs with no Gram-staining organisms. Cultures not attempted.

32. Submaxillary gland containing a lesion similar to that in No. 30, measuring  $2.1 \times 1.4$  cm.; granules slightly gritty. Cultures sterile, excepting a few moulds.

33. Lesions in the floor of the mouth consisting of two nodules the size of thrush's eggs. They were composed of soft yellowish tissue, purulent in the central portion and containing numerous granules. The whole was surrounded by fibrous tissue. Two glands at the angle of the jaw each showed small nodules of similar material to the above. There was also a caseo-calcareous area in each gland, from one of which pure cultures of tubercle bacilli were obtained. Cultures positive.

34. The posterior portion of the tongue was infiltrated with fibrous translucent nodules containing soft tissue with numerous granules. The nodules were aggregated at the sides into dense fibrous areas, over which the epithelium was ulcerated, and they extended deeply into the muscle of the blade and roots. A submaxillary gland contained a similar nodule 1 cm. in diameter. Cultures positive.

35. Submaxillary gland showed on section a nodule measuring  $4.2 \times 3 \times 3.5$  cm. This consisted of soft translucent tissue closely beset with opaque yellowish foci; microscopically typical granules. Cultures positive.

36. Pharyngeal gland contained small areas consisting of granulomatous tissue with yellowish granules surrounded by fibrous tissue. Submaxillary gland was almost replaced by a nodule  $4 \times 2$  cm. similar to the above. Cultures positive.

37. Portion of cheek muscle containing numerous nodules of soft tissue beset with yellow foci and surrounded by much fibrous tissue. The largest measured 2.2 cm. Submaxillary gland contained a similar nodule 1.3 cm. in diameter. Cultures positive.

38. Posterior two-thirds of tongue showed beneath the epithelium numerous soft grey nodules with yellowish foci surrounded by fibrous

tissue and ranging up to 1 cm. in diameter. They extended into the muscle, but not deeply. Four had ulcerated through the epithelium, forming projecting tumours. Submaxillary gland showed an area containing three nodules similar to the above, from .4 to .8 cm. in diameter. Cultures positive.

39. Portion of palate showed numerous irregular ulcers up to  $4 \times 1.5$  cm. in area. Beneath the epithelium were numerous soft grey nodules with yellowish granules. The nodules were occasionally purulent in the centre, and were surrounded and separated by fibrous tissue. Submaxillary gland contained similar nodules up to 1 cm. in diameter. A second gland was almost replaced by nodules becoming purulent. Pharyngeal gland contained similar nodules. Cultures from both pharyngeal and submaxillary glands positive.

40. Pharyngeal gland was replaced by a mass, 4 cm. in diameter, of soft, streaky yellowish substance exuding pus when squeezed and containing numerous granules. The gland had ulcerated through the pharyngeal wall, forming a prominent fungating tumour. Cultures positive.

#### *Cultures.*

Cultures have been attempted from all but a few of the forty cases, and have been successful in twenty-three, where apparently identical cultures were obtained. In the unsuccessful cases the cultures have remained sterile or have become overgrown.

An emulsion was made, either in a small test tube or in a mortar, of granulation tissue or pus obtained from the centre of a nodule, after searing the surface in the usual way. This emulsion was spread over a series of plates of agar made with veal broth, reaction + 10 (Eyre's method). After 24 hours' incubation at 37° C. translucent circular colonies with a regular outline were formed, ranging up to 1.5 mm. in diameter, coherent and bluish-grey by transmitted light. Generally the colonies were very numerous, occasionally sparse—only one or two to a plate. In sub-culture on slant agar a non-characteristic growth is produced, resembling somewhat that of *B. typhosus*. The cohesiveness of the primary colonies is quickly lost. Microscopically on this medium the organism grows in the form of a short bacillus or coeco-bacillus, with occasional long forms. The bacillus does not retain the stain by Gram's method; it stains best with weak carbol-fuchsin. It is non-motile. Viability is not great. The numbers of organisms capable of producing colonies rapidly diminish in emulsion of tissue kept in the ice-chest,

and sub-cultures in series must be made every four or five days. In glucose agar shake tubes growth occurs in the depth as minute colonies, but just below the surface of the medium the colonies, when not too numerous, grow much larger and assume a rosette form. Microscopically such colonies are composed of a mass of long, tangled, unbranched filaments, not Gram-staining, with a variable number of smaller curved bacilli and circular bodies. On transplantation to slope agar the ordinary short bacillary form is resumed.

Several strains (Nos. 19, 26, 28, 29, and 35) have been tested on various other culture media, with the following results:

Solidified egg medium: growth sparse, in the form of circular slimy colonies consisting of involution forms. They retain their vitality, and have been transplanted after two or more months.

Broth (+ peptone): uniform turbidity and surface scum.

1 % peptone salt water: slight growth.

10 % peptone salt water: uniform turbidity.

Lactose litmus agar (Würtlz): reddened in 48 hours.

Neutral red agar plates + lactose or dextrose: red colonies.

Litmus milk: acidity, no clot.

Glycerin-litmus milk: acidity and clot.

Bile salt agar plates: colonies either moist or coherent.

Potato (acid) pure: discrete moist, slimy, grey colonies after 12 days.

Microscopically either long threads or irregular involution forms.

Potato alkaline to litmus: as above.

Glycerin-agar: moist, slimy translucent layers.

Solidified serum: thin layers as above.

Solidified glycerin serum: thin layers as above.

Veal extract (— peptone): no uniform turbidity; flakes of growth which deposit on the bottom or sides of the tubes, leaving medium clear.

Serum water + litmus (Hiss): no change in reaction. Growth feeble.

#### *Inoculation Experiments.*

On June 16th, 1914, a calf was inoculated subcutaneously on the left side of the neck with the growth from six agar tubes of 24-hours-old culture from No. 11. Total duration of sub-cultivation 33 days. A fluctuating tumour rapidly formed, and there was a slight rise of temperature for a few days. A second inoculation was performed, July 7th, 1914, on the other side of the neck with the 24-hours-old culture from four agar tubes of No. 2. There was a slight rise of temperature and

a local reaction which ultimately disappeared without leaving a trace behind—a result possibly due to the immunising effect of the first inoculation. The animal was killed in good condition on August 8th, 1914. There was a hard subcutaneous tumour on the left side of the neck about the size of a partridge's egg. On section it was composed of a collection of soft greyish nodules with slightly yellowish points surrounded by dense fibrous tissue. The prescapular gland was normal, and the animal was otherwise healthy.

Microscopically the local lesion showed numerous granules similar to those found in the natural disease, but rather smaller, more irregular, and less coherent. Under a higher magnification the granules were composed of masses of minute Gram-negative bacilli, around which were typical small refractile clubs. In histological sections the appearances were identical with those in cases of the natural disease.

Two pigs were each fed and inoculated with emulsions of actinomycotic tissue from Nos. 6 and 7. No disease was produced.

One pig was inoculated subcutaneously with culture; when killed no lesion was found.

A monkey was inoculated subcutaneously with  $1\frac{1}{2}$  agar cultures of No. 11 24 hours old. The animal remained healthy, and no lesion was found.

A monkey was inoculated subcutaneously with culture from eight agar tubes four days old, of No. 19, on November 23rd, 1914. The animal was alive and healthy on January 11th, 1915.

Guinea-pigs inoculated intraperitoneally with  $\frac{1}{4}$  to 1 slant agar growth became ill after the inoculation. They either died in from 1–3 days or after a brief illness recovered and survived. Flakes of pus were found scattered about the peritoneal cavity. No granules or clubs were found.

## GROUP II.

In this group are four cases, in each of which the lesions were in the inferior maxillary bone. The bone was expanded and occupied by a mass of granulation tissue containing large numbers of the characteristic granules. Microscopically these granules were composed of masses of Gram-staining organisms comprising long branched filaments, chains of coccal bodies, and short bacilli. Around the periphery of the granules were numerous clubs staining with eosin.

The investigation of the biological properties of the organisms concerned in the production of the lesions has not yet been completed.



## SUMMARY.

Actinomycotic lesions in tissues from a consecutive series of forty-four oxen slaughtered in this country have been examined and compared with fifty specimens imported chiefly from Argentina.

The British cases can be divided into two groups, according as the specific granules do not or do contain Gram-staining organisms.

The first group, characterised by the presence of granules consisting of clubs without Gram-staining organisms, contains 40 cases. In these the lesions were situated in the cheek, palate, tongue, or lymphatic glands in relation to the mouth and pharynx. From twenty-three of the forty cases, cultures of a non-Gram-staining organism were obtained, which were identical in their characters on nutrient agar plates and in shake glucose agar tubes; and five of the strains were found identical when tested on a large series of differential media. One of the cultures was inoculated into a calf, and produced a local lesion with the characters of a natural lesion.

This first group shows complete identity in the histological features and in the anatomical distribution of the disease with Actinobacillosis described by Lignières and Spitz in Argentina. The bacillus obtained has the characters of the *Actinobacillus*, except that the production of typical granules in the peritoneal cavity of guinea-pigs, described by Lignières and Spitz, has not been demonstrated.

All the imported specimens which were examined microscopically exhibited the characters of the lesions of this first group, but the causal organism was not obtained in pure culture.

The second group contains the remaining four British cases. The specific granules were composed of clubs and Gram-staining organisms, including branching filaments. The lesions were situated in each case in the inferior maxilla. The investigation of the biological characters of the organisms concerned is still in progress.

These results show that Actinobacillosis is widespread in the world, and forms a considerable proportion of the cases of disease in oxen known under the name of Actinomyces.

THE INFLUENCE OF THE AGE OF THE PARENT  
AT THE BIRTH OF OFFSPRING ON THE AGE  
AT WHICH THEY ARE ATTACKED BY SOME  
OF THE ZYMOTIC DISEASES, WITH SPECIAL  
REFERENCE TO THE EPIDEMIOLOGY OF  
SCARLET FEVER.

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(With 2 Charts.)

IN a previous paper (1912) a rough analysis of certain statistical data was made which seemed to show that the age of the parent at the birth of the offspring was a factor in its susceptibility to the Zymotic Diseases.

In the present paper it is proposed to consider the existence of such a possibility in more detail with special reference to the fallacies that are likely to arise.

The official sources of information are twofold, firstly the Death Returns published by the Registrar General and secondly the Notification of Infectious Disease. Most of the data dealt with have been collected through the latter of these channels.

The first point is, to what extent do these returns fail to reflect the actual prevalence of the disease here considered? The question may be divided into errors of excess and defect, Scarlet Fever being taken as an example.

(a) *Errors of excess.*

The most comprehensive figures dealing with this point are to be found in the Metropolitan Asylums Board's Reports from the year 1902

to the present. The actual data for the ten years 1903 to 1912 are as follows:

	Estimated population to middle of year	Notifications			Percentage of Notifications admitted to hospital			Cases notified as Scarlet but suffering from other diseases	Cases notified as Diphtheria but suffering from other diseases	Cases notified as Enteric but suffering from other diseases
		Scarlet Fever	Diphtheria (including membranous croup)	Enteric Fever	Scarlet Fever	Diphtheria	Enteric Fever			
1901	4,544,983	—	—	—	—	—	—	—	—	—
1903	4,613,812	12,531	7739	2339	83.8	80.3	51.8	701	873	308
1904	4,648,950	13,439	7219	1896	84.5	79.5	51.7	806	902	268
1905	4,684,794	19,461	6482	1552	88.6	82.1	51.4	975	908	247
1906	4,721,217	20,329	8045	1600	88.5	79.7	55.1	932	959	235
1907	4,758,218	25,925	8771	1394	89.4	83.2	51.5	1670	1180	222
1908	4,795,757	22,071	8002	1357	90.9	84.1	50.4	1202	1159	199
1909	4,833,938	17,254	6679	1043	90.2	85.7	49.3	1132	930	202
1910	4,872,702	10,509	5494	1284	88.9	84.8	51.7	918	599	168
1911	4,521,301	10,483	7385	1022	89.2	85.0	50.6	843	783	187
1912	4,519,754	11,321	7106	705	90.6	86.7	52.9	672	775	153

From these figures, it is seen that the discrepancies between the notifying Doctor and the Receiving Officer are:

	Scarlet Fever %	Diphtheria %	Enteric Fever %
1903	6.97	14.05	25.55
1904	7.10	15.71	27.34
1905	5.65	17.06	30.95
1906	5.18	14.96	26.64
1907	7.21	16.17	30.92
1908	5.99	17.22	29.29
1909	7.27	16.25	39.30
1910	9.83	12.86	25.30
1911	9.02	12.47	36.17
1912	6.55	12.58	40.02

In Scarlet Fever, the particular disease under consideration, the percentage varies from 5-9. As to whether this is a true error of excess cannot be decided in the absence of definite bacteriological tests, but it may be taken to represent it rather closely, for in those hospitals where little or no attempt is made to isolate doubtful cases, approximately 5 % of the total number of admissions develop the disease during the course of their stay.

If the numbers of cases said to be wrongly diagnosed are correlated with the total numbers, the population being made constant, the result is  $r = -.2974 \pm .1248$  for Scarlet Fever,  $-.0163 \pm .1349$  for Diphtheria, and  $-.2513 \pm .1239$  for Enteric Fever.

That is to say, as the number of cases goes up, the percentage error goes down. It would seem to suggest that certain fairly constant numbers of cases are returned each year, suffering from other diseases than that with which they are labelled. It is obvious that if one takes samples of a universe and notes the frequencies of *A*'s and *B*'s and adds to both groups a certain number of *C*'s in fairly equal proportions, then some correlation between *A* and *B* must exist depending on the proportion of *C*'s. Hence if we correlate the prevalence of Scarlet Fever and Diphtheria in different areas for any year, counting along with each, other diseases, a relationship must exist. In effect, we find<sup>1</sup> that the correlation between Scarlet Fever and Diphtheria

in 1912 for the County Boroughs of England	$r = \cdot 1067 \pm \cdot 0831$
„ 1911 „ „ „ „	$r = \cdot 1796 \pm \cdot 0842$
„ 1912 Rural Counties of England	$r = \cdot 2454 \pm \cdot 0875$
„ 1911 „ „ „ „	$r = \cdot 2644 \pm \cdot 0808$
„ 1911 for the American Cities (Mass.)	$r = \cdot 0696 \pm \cdot 1012$

<sup>1</sup> The example was worked out in class at the Lister Institute.

TABLE I. *Theoretical distribution of Scarlet Fever, according to age of*

Age of attack	Age of parent at birth															
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
0	·5	1	2	3	3·5	4·5	5·5	5·5	5·5	5·5	5·5	5	5	4·5	4·5	4
1	1	3	7	10·5	12·5	15	17·5	18	18·5	18	17·5	17	16	15·5	14·5	14
2	2	7·5	14·5	22	26	31	35·5	37	37·5	36·5	35·5	34·5	33	32	30	28·5
3	3	10·5	20·5	30·5	36	43·5	49·5	51·5	52	51	49·5	48	46	44	41·5	39·5
4	3·5	12·5	24	35·5	42	51	58	60·5	60·5	59·5	58	56	54	51·5	48·5	46
5	4	13	25	37	44	53	60·5	63	63	61·5	60·5	58	56	53·5	50·5	47·5
6	3·5	12	23·5	35	41	50	56·5	60	59	58	56·5	54·5	52·5	50	47	44·5
7	3	10·5	20	30	35	42·5	48	49·5	50·5	49·5	48	46·5	44·5	42·5	40	38
8	2·5	9	17·5	25·5	30·5	37·5	41·5	43·5	43·5	42·5	41·5	40	38·5	36·5	34·5	32·5
9	2	7·5	14·5	21·5	25·5	31	35	36·5	36·5	35·5	35	33·5	32·5	31	29	27·5
10	2	6·5	12·5	18	21·5	26	29·5	30·5	31	30	29·5	28	27	26	24·5	23
11	1·5	5	10	14·5	17·5	21	23·5	24·5	24·5	24	23·5	22·5	21·5	20·5	19·5	18·5
12	1	4	8	12	14	16·5	19	20	20	19·5	19	18	17·5	16·5	15·5	14·5
13	1	3·5	6	9·5	11	13·5	15·5	16	16	15·5	15	14·5	14	13·5	12·5	12
14	·5	2	4	6	7·5	8·5	10	10·5	10·5	10	10	9·5	9	8·5	8	7·5
15	·5	2	3·5	5	6	7	8	8·5	8·5	8·5	8	7·5	7·5	7	6·5	6·5
16	·5	1·5	3	4	5	6	6·5	7	7	6·5	6·5	6·5	6	5·5	5·5	5
17	·5	1	2·5	3·5	4	4·5	5·5	5·5	5·5	5·5	5·5	5	5	4·5	4·5	4
18	·5	1	2	3	3·5	4	4·5	5	5	4·5	4·5	4·5	4	4	4	3·5
19	·5	1	2	2·5	3	3·5	4	4·5	4	4	4	4	3·5	3·5	3·5	3
20	—	·5	1·5	2	2·5	3	3·5	3·5	3·5	3·5	3	3	3	3	2·5	2·5
Totals	33·5	114·5	223·5	330·5	391·5	472·5	537	560·5	562	549	536	516	496	473·5	446·5	422



Thus in the American Cities (Mass.) there is no association, in the County Boroughs it is twice its probable error and in the Rural Districts highest of all. This would seem to suggest that in England some other diseases are indiscriminately returned as either Scarlet Fever or Diphtheria. The following figures taken from the Metropolitan Report for 1906 support this contention.

	Scarlet Fever 17,983	Diphtheria 6319
Number of cases isolated ...		
Number found to be suffering from other diseases ...	932	959
The above include Measles ...	93	47
Tonsillitis, etc.	178	661

Thus a proportion of the rate in each case consists of conditions which might be included in either; hence the correlations found may be due to the excessive prevalence of simple inflammatory conditions of the respiratory passages so common in these Islands. There is no evidence to show in what way this error will distribute itself with respect to age at birth, and if it is assumed that it takes the form of

*parent at birth and age of attack—the parent being alive—original number 10,000.*

Age of parent at birth																Totals
33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	
4	4	3·5	3	2·5	2·5	2	1·5	1·5	1	1	·5	·5	·5	—	—	93·0
13	12·5	11·5	10	8·5	8	6·5	5	4·5	3·5	2·5	2	1·5	1	·5	·5	306·5
27	25	23	21	17·5	16	13	10·5	9	7	5·5	4·5	2·5	1·5	1	·5	627·5
37·5	35	32	29	24·5	22	18	14·5	12·5	9·5	7·5	6	4	2·5	1·5	1	873·5
43·5	41	37·5	33·5	28·5	25·5	21	17	14·5	11	9	7	4·5	3	1·5	1	1019·0
45	42	39	35	29·5	26·5	22	17·5	15	11·5	9	7	4·5	3	1·5	1	1059·0
42	39·5	36	32·5	27·5	24·5	20·5	16	14	10·5	8·5	6·5	4	2·5	1·5	1	990·5
35·5	33·5	31	27·5	23·5	21	17·5	14	12	9	7	5·5	3·5	2	1·5	·5	842·5
30·5	28·5	26·5	23·5	20	18	15	12	10	7·5	6	4·5	3	2	1	·5	725·0
25·5	24	22	20	16·5	15	12·5	10	8·5	6·5	5	4	2·5	1·5	1	·5	609·0
21·5	20	18·5	16·5	14	12·5	10	8·5	7	5·5	4	3·5	2	1·5	·5	·5	511·5
17·5	16	15	13·5	11	10	8	6·5	6	4	3·5	2·5	1·5	1	·5	·5	409·0
14	13	12	10·5	9	8	6·5	5	4·5	3·5	2·5	2	1·5	1	·5	·5	329·0
11	10·5	9·5	8·5	7	6·5	5·5	4	3·5	2·5	2	1·5	1	·5	·5	—	263·0
7	6·5	6	5·5	4·5	4	3·5	2·5	2·5	2	1·5	1	·5	·5	—	—	169·5
6	5·5	5	4·5	3·5	3·5	2·5	2	2	1·5	1	1	·5	·5	—	—	139·5
4·5	4·5	4	3·5	3	2·5	2	2	1·5	1	1	·5	·5	·5	—	—	113·0
4	3·5	3	3	2·5	2	2	1·5	1	1	·5	·5	·5	—	—	—	91·5
3·5	3	3	2·5	2	2	1·5	1	1	1	·5	·5	·5	—	—	—	79·5
3	2·5	2·5	2	2	1·5	1·5	1	1	·5	·5	·5	—	—	—	—	69·0
2·5	2	2	1·5	1·5	1·5	1	1	·5	·5	·5	·5	—	—	—	—	55·5
398	372	342·5	306·5	258·5	233	192	153	132	100	78·5	61·5	39	25	13	8	9376

a dilution of material, then any correlation found will be reduced according to the formula

$$\frac{r_2}{r_1} = \frac{n_1}{n_1 + n_2},$$

where  $n_1$  = numbers of correlated pairs,  $n_2$  = numbers of non-correlated pairs.

(b) *Errors of defect.*

Another source of fallacy arises from the manner in which the material is collected, as it is usual to obtain information only concerning the actual occupants of the house in which the disease occurs. Hence a child of 13 born by a woman of 45 should have a mother aged 58 if alive and it is obvious that there is a certain defect owing to the mothers of young adults born late in life having died. Some idea of this can be obtained by calculations based on the 1901-11 life table. The frequency that should be observed where the mother is living, is given on a basis of 10,000 cases of Scarlet Fever, in Table I.

The correlation which must necessarily occur between age of mother at birth and age of child when attacked is  $-.016$ , that is to say, that any positive association in numbers over 1000 is probably significant of some bias. Of course no record was obtained of mothers who had deserted or left their children, this error is small and probably has no constant tendency.

(c) *Errors dependent on the source of infection.*

This source of fallacy is still more subtle and difficult to gauge and may be stated as follows:

Infection can be divided into two great sources:

- (a) General; such as all are equally exposed to, and
- (b) Family; that is, the risk that arises of one member of a family infecting another.

In Scarlet Fever, this source accounts for over 13 % if we assume that all cases other than the primary one are due to infection in this way.

Now it is obvious that home infection will vary according to the size of the family, their respective ages, and the extent of overcrowding. The necessity of considering these three points is fairly obvious. The following figures taken from Dr Chalmers' paper (1913) show that overcrowding may not only favour spread but may prejudice recovery.

*Death rates per 1000 of similar ages, 1—5 years (Males).*

				Measles	Whooping Cough	Scarlet Fever	Diphtheria
1	Apartment	...	...	8.6	4.36	1.26	2.62
2	Apartments	...	...	5.9	2.79	.97	1.56
3	„	...	...	2.85	1.42	.22	1.87
4	„	and upwards		1.01	.67	—	1.01

The fall in the death rates is very apparent.

It is of interest to note, that although the same facts are fairly constant for Measles and Whooping Cough, some variation with respect to Scarlet Fever is noted when other age periods are considered.

*Males—Death rates.*

				0—1 years	1—5 years	6—10 years	All ages
1	Apartment	...	...	.28	1.26	.26	.23
2	Apartments	...	...	.32	.97	.36	.18
3	„	...	...	.32	.22	.19	.06
4	„	and upwards		—	—	.77	.08

*Females—Death rates.*

				0—1 years	1—5 years	6—10 years	All ages
1	Apartment	...	...	.41	1.03	.51	.20
2	Apartments	...	...	.32	.95	.39	.19
3	„	...	...	.33	.72	.24	.08
4	„	and upwards		—	.32	.10	.03

Hence overcrowding must be a factor in the spread of the disease.

The relationship is however more complex than appears from “a priori” reasoning. The following data were obtained in the Urban District of Barking Town with a population of 33,000 and give the size of family in which cases of Scarlet Fever occurred, when all are considered and when the initial case only is counted, that is to say, if several cases occur in one family, it is counted once only. These distributions are compared with (*a*) families which contain a child of school age, and (*b*) families which contain a child in its fifth year of age, the mean age being 5 years and 8 months. (Table II.)

It is seen that taking all cases the large families are unduly represented, but that taking each family once only, the distribution agrees very closely with that of families which contain a child in its 5th year. Now the mean age of attack for Scarlet Fever was 7.2 years. Hence this series of observations would seem to suggest that if the spread in the family itself be ignored, small families are more likely to be attacked than large ones. Such a conclusion might be explained in one of two ways, either small families are biologically different from large, or, in

consequence of better medical supervision a smaller number of cases are overlooked. Pursuing this line of inquiry in more detail, and with the special object of examining the relationship of Scarlet Fever, and the mean size of families, the following data relative to the Rural districts of the Eastern Counties were collected.

TABLE II.

*Scarlet Fever and size of family in Barking Town.*

Size of family	Number in which a case of Scarlet Fever occurred		Proportion	Number which contain a child in 6th, 9th or 13th year		Proportion	Number which contain a child in 6th year only		Proportion
	All cases	(1st invasion only)							
1	11	11	·11579	70	·05414	33	·07174		
2	19	16	·16842	117	·09049	97	·21087		
3	21	16	·16842	239	·18484	105	·22826		
4	32	22	·23158	193	·14927	67	·14564		
5	21	11	·11579	186	·14385	60	·13043		
6	10	6	·06316	121	·09358	31	·06739		
7	9	6	·06316	75	·05800	23	·04999		
8	2	1	·01053	77	·05955	16	·03478		
9	10	4	·04211	60	·04640	8	·01739		
10	1	1	·01053	34	·02630	7	·01522		
11	—	—	—	25	·01933	7	·01522		
12	—	—	—	10	·00774	4	·00870		
13	—	—	—	9	·00696	2	·00435		
14	—	—	—	13	·01005	—	—		
15	3	1	·01053	3	·00232	—	—		
16	—	—	—	1	·00077	—	—		
<hr/>									
	139	95	1·000	1293	1·000	460	1·000		
Notation		$X_1$		$X_2$		$X_3$			
		$X_{12}^2 = 80·3181,$		$P = ·000034,$					
		$X_{13}^2 = 22·4014,$		$P = ·6944.$					

If 3 be raised from 460 to 1293

$$X_{13}^2 = 35·5324$$

$$P = ·1526.$$

Hence 1 and 3 are similar distributions.

Mean age of attack for Scarlet Fever 7·22 years.

The Rural districts were chosen rather than the Urban, because the proportion of the rate dependent on spread in the family would presumably be greater owing to chances of infection from a general source being less. That such is the case, is borne out by the fact that taken as a whole the rate for a Rural population is less than for the Urban areas; it must also be remembered that medical attention is more expensive in sparsely populated districts than in the crowded centres.



The factors considered were as follows:

(1) Population. This is desirable as a spurious correlation is said to exist if indices are used and also it is desirable to give the proper significance to large and small districts.

(2) Acres per family. This is necessary because the chances of infection from a general source must be considered.

(3) Proportion under 10 years of age, because the incidence of Scarlet Fever is mainly on those below that age.

(4) Percentage, with more than two per room, because the greater the degree of overcrowding, the closer the contact between individual members of the family and the greater chance of spread in the family itself.

The data are as follows:

TABLE III.

*Rural Districts of Eastern Counties and incidence of Scarlet Fever.*

District	Population	Acres per family	Mean size of family	Proportion 10 years of age and under	Mean rates for years 1911-12-13		Percentage living more than two per room
					Scarlet Fever	Diphtheria	
<i>Bedford</i>							
Ampthill .....	19,540	13.56	3.91	19.19	2.01	0.43	3.2
Bedford .....	19,213	20.16	3.92	19.26	1.96	0.89	4.0
Biggleswade....	21,933	10.55	4.07	19.62	1.96	0.23	5.5
Eaton Bray ...	3,892	13.94	3.99	21.35	2.50	2.37	3.2
Eaton Socon ..	3,542	20.21	4.06	22.53	2.09	0.66	3.0
Luton .....	9,474	16.2	4.21	21.29	0.91	0.63	4.7
<i>Cambridge</i>							
Caxton .....	7,769	25.4	4.13	19.93	0.59	0.13	4.7
Linton .....	10,546	18.1	3.93	19.07	2.46	1.05	4.3
Melbourne .....	8,536	18.6	3.87	19.62	1.52	0.42	4.5
Newmarket ....	19,970	16.9	4.15	22.39	4.62	1.20	8.0
Swavesey .....	2,559	21.0	3.74	19.85	6.04	0.46	5.0
<i>Essex</i>							
Billericay .....	22,091	11.7	4.18	17.54	1.34	1.18	3.5
Braintree .....	18,510	13.2	3.86	17.55	1.68	0.53	3.7
Belehampt.....	4,653	21.6	3.81	17.88	1.14	0.21	3.5
Bumpstead ....	2,601	18.1	3.92	20.68	0.90	5.86	6.4
Chelmsford ....	23,065	14.9	4.08	19.71	1.23	1.10	2.5
Dunmow .....	16,134	18.6	3.92	19.17	1.97	0.41	3.4
Epping .....	14,115	12.2	4.30	20.23	2.87	1.47	4.3
Halstead .....	10,353	14.8	3.92	19.23	0.90	0.26	2.9
Lexden .....	19,832	14.4	4.07	20.37	1.03	0.76	4.6
Maldon .....	16,367	20.4	4.02	21.03	1.60	0.91	2.8
Ongar .....	10,723	20.3	4.25	20.71	1.28	0.28	4.5
Orsett .....	18,938	7.7	4.45	22.89	3.87	0.78	6.6
Romford .....	26,194	5.43	4.47	22.62	1.81	0.68	4.6
Rochford .....	18,880	13.0	4.20	20.76	1.28	1.46	4.0
Saffron Walden	10,816	21.8	3.92	19.53	1.88	1.14	4.4
Stansted .....	7,090	13.4	4.12	20.38	0.23	0.24	3.6
Tendring .....	22,171	14.1	4.01	20.58	0.59	0.28	4.1

TABLE III—(continued).

*Rural Districts of Eastern Counties and incidence of Scarlet Fever.*

District	Population	Acres per family	Mean size of family	Proportion 10 years of age and under	Mean rates for years 1911-12-13		Percentage living more than two per room
					Scarlet Fever	Diphtheria	
<i>Huntingdon</i>							
Huntingdon....	6,938	32.97	4.05	20.32	0.92	0.19	4.0
Norman Cross..	5,624	23.36	4.31	21.10	0.89	0.79	3.8
St Neots .....	7,227	27.78	3.95	18.78	2.35	0.56	3.5
St Ives.....	9,701	18.29	3.85	20.0	0.62	0.38	2.8
<i>Lincoln</i>							
Bourne .....	13,391	25.57	4.15	20.80	1.19	0.71	4.6
Branston .....	14,913	21.97	4.24	19.50	1.04	0.85	3.0
Caistor .....	13,146	38.55	4.19	20.59	1.65	0.53	2.2
Glandford Brigg	29,895	17.99	4.34	23.67	1.20	0.56	3.2
Gainsborough ..	14,743	26.38	4.10	20.73	1.70	0.97	2.7
Grimsby.....	10,629	19.74	4.16	21.46	1.14	1.84	5.1
Grantham .....	12,257	30.59	4.23	21.14	1.44	1.41	4.8
Horncastle .....	13,102	35.83	4.13	20.88	1.35	0.56	3.2
Louth .....	18,285	34.03	4.02	19.64	0.60	0.45	2.4
Spilsby .....	21,012	24.45	3.94	19.43	1.51	0.90	2.5
Sleaford .....	17,269	31.65	4.23	20.38	1.95	0.64	4.1
Spalding .....	13,186	23.56	4.33	22.40	1.68	0.12	3.6
Welton .....	11,111	31.16	4.13	20.30	2.25	1.02	2.4
<i>Norfolk</i>							
Aylsham.....	17,383	16.88	4.13	20.92	2.55	0.62	5.8
Blofield .....	12,201	17.3	4.10	17.95	2.02	1.09	3.9
Depwade .....	19,909	16.3	4.01	20.25	0.90	0.48	3.2
Doeking .....	17,101	21.2	4.01	19.61	0.86	0.61	5.5
Downham .....	15,618	21.8	4.10	20.60	0.91	0.10	4.0
Forehoe .....	11,390	13.7	3.99	20.68	1.02	0.90	3.9
Flegg .....	10,027	12.1	4.13	23.06	1.73	0.87	3.2
Henstead .....	10,275	17.2	4.14	19.21	4.18	1.52	3.3
Freebridge, Lynn	12,140	25.4	4.07	19.53	1.13	0.33	4.4
Erpingham .....	17,272	15.5	4.16	20.24	1.28	1.20	4.8
Loddon .....	12,571	20.1	4.12	20.68	1.65	1.41	4.3
Marchland .....	12,518	18.0	4.05	21.01	1.10	0.51	3.6
Mitford.....	18,733	22.0	3.9	20.37	1.00	0.86	4.1
Smallburgh .....	12,434	18.8	3.99	22.21	1.29	1.14	5.4
St Faith's .....	10,864	19.6	4.09	18.62	2.10	1.02	4.9
Swaffham.....	7,561	39.5	3.99	21.00	0.92	0.31	2.9
Thetford.....	10,076	39.1	4.10	20.95	0.53	0.10	4.5
Wakingham....	17,266	19.4	4.15	23.87	3.11	1.91	5.3
Wayland .....	14,509	19.5	3.94	19.13	2.49	0.23	3.1
Mean.....	13,778	20.42	4.088	20.41	1.66	—	4.04
Standard deviation.....	57.7105	7.6553	0.1591	1.3510	1.0001	—	1.0856
Notation	$r_6$	$r_4$	$r_2$	$r_3$	$r_1$	$r_5$	
Coefficients							
	$r_{12} = -0.083 \pm 0.035,$		$r_{24} = -0.2971 \pm 0.0763,$		$r_{16} = -0.0412 \pm 0.0335,$		
	$r_{13} = -0.823 \pm 0.030,$		$r_{25} = -0.5115 \pm 0.0618,$		$r_{26} = -0.251 \pm 0.036,$		
	$r_{14} = -0.1717 \pm 0.011,$		$r_{34} = -0.2821 \pm 0.0770,$		$r_{36} = -0.0971 \pm 0.0829,$		
	$r_{15} = -0.3531 \pm 0.0732,$		$r_{35} = -0.3055 \pm 0.0758,$		$r_{46} = -0.2999 \pm 0.0761,$		
	$r_{23} = -0.2012 \pm 0.0803,$		$r_{45} = -0.0344 \pm 0.0836,$		$r_{56} = -0.0918 \pm 0.0830,$		
Partial coefficients							
	$r_{12} = -0.083 \pm 0.035,$		${}^3r_{12} = -0.0562 \pm 0.0834,$		${}^{34}r_{12} = -0.1104 \pm 0.0826,$		
	${}^{345}r_{12} = -0.3565 \pm 0.0730,$						
	${}^{234}r_{13} = -0.4819 \pm 0.0642,$		${}^{235}r_{14} = -0.3013 \pm 0.0761,$		${}^{245}r_{13} = -0.1034 \pm 0.0827,$		

In two instances only do we find that the association between Scarlet Fever and these variables is significant, namely, with acres per family and overcrowding, that is, greater facilities for the spread of the disease, whether in the general or particular sense, increases the rate. In forming partial correlations, population can be ignored, as in one instance only, namely with acres per family, is the coefficient more than twice its error. Taking the remaining five we have:

(1) Scarlet Fever rate and mean size of family  $r_{12} = -\cdot0383 \pm \cdot0835$ .

(2) With proportion of children under 10 years constant

$${}_3r_{12} = -\cdot0562 \pm \cdot0834.$$

(3) With proportion of children under 10 and acres per family constant

$${}_{34}r_{12} = -\cdot1104 \pm \cdot0826.$$

(4) With proportion of children under 10, acres per family and proportion living more than two per room constant

$${}_{345}r_{12} = -\cdot3565 \pm \cdot0730.$$

From these figures we can conclude that, with a population of constant susceptibility and constant chance of infection both at home and abroad, the smaller families are much more likely to be attacked than large ones. It is to be observed as each additional factor is taken into consideration the association becomes more marked.

If we take the Scarlet Fever rate with proportion of children under ten years, with chance of infection constant we have  ${}_{45}r_{13} = -\cdot0876 \pm \cdot0829$  and with size of family under similar conditions  ${}_{45}r_{12} = -\cdot3518 \pm \cdot0731$ , which also suggests that size of family, irrespective of age or chance of infection, influences the rate. The remaining partial coefficients found are such as "a priori" reasoning would suggest. Thus both in Urban Districts, when the initial invasion, and in Rural Districts when all cases are considered, there is reason to believe that Scarlet Fever is likely to come to the notice of the authorities in small families rather than in large. In so far as Scarlet Fever has been for many years a mild disease and the symptoms very indefinite, the result found does suggest that an error of omission exists and is greatest when the financial position of the family is straitened. It must, however, be remembered that the mean age of the mother at birth of offspring does not vary materially with the size of completed families and that small families are more closely centred round the 28th year than large ones, hence if age at birth does influence susceptibility, that is, those born at maturity

react more characteristically than those born at the extreme of the reproductive epoch, then we should expect the results that have already been found. Both of the explanations may be correct, though the weight of evidence is in favour of a large error of omission. The following data rather lend support to this view.

Inquiry through the Education Act (Administrative Provisions), 1907, was made from several areas as to the number of children found to be actually suffering or recently recovered from Scarlet Fever during the Routine Medical Inspection. (The criteria on which this opinion was based were desquamation with a recent history of illness.) The groups selected were those entering on or finishing their education, that is, approximately, the 5th and 13th years of age. The proportion of each was about equal. The figures were as follows:

Number examined, 58,936. Number found desquamating and with a history of a recent illness or actually suffering from Scarlet Fever 13. Number of cases of Scarlet Fever notified 5120. Number of same age as children examined 726.

Now if thirteen were actually found and the search conducted for two-thirds of the year (Saturdays, Sundays and Holidays omitted) the actual number for a complete year is 19·5. If we agree that the illness, or its subsequent desquamation, can be easily detected for an average period of fourteen days, then the number that must have existed will be  $20 \times 26 = 520$ . The actual number recorded was 726. From a practical knowledge of Medical Inspection of School Children and its deadening tendency, I think this calculation rather underestimates the actual error of omission. Hence an error of 40 % in defect will probably not be very far from the truth.

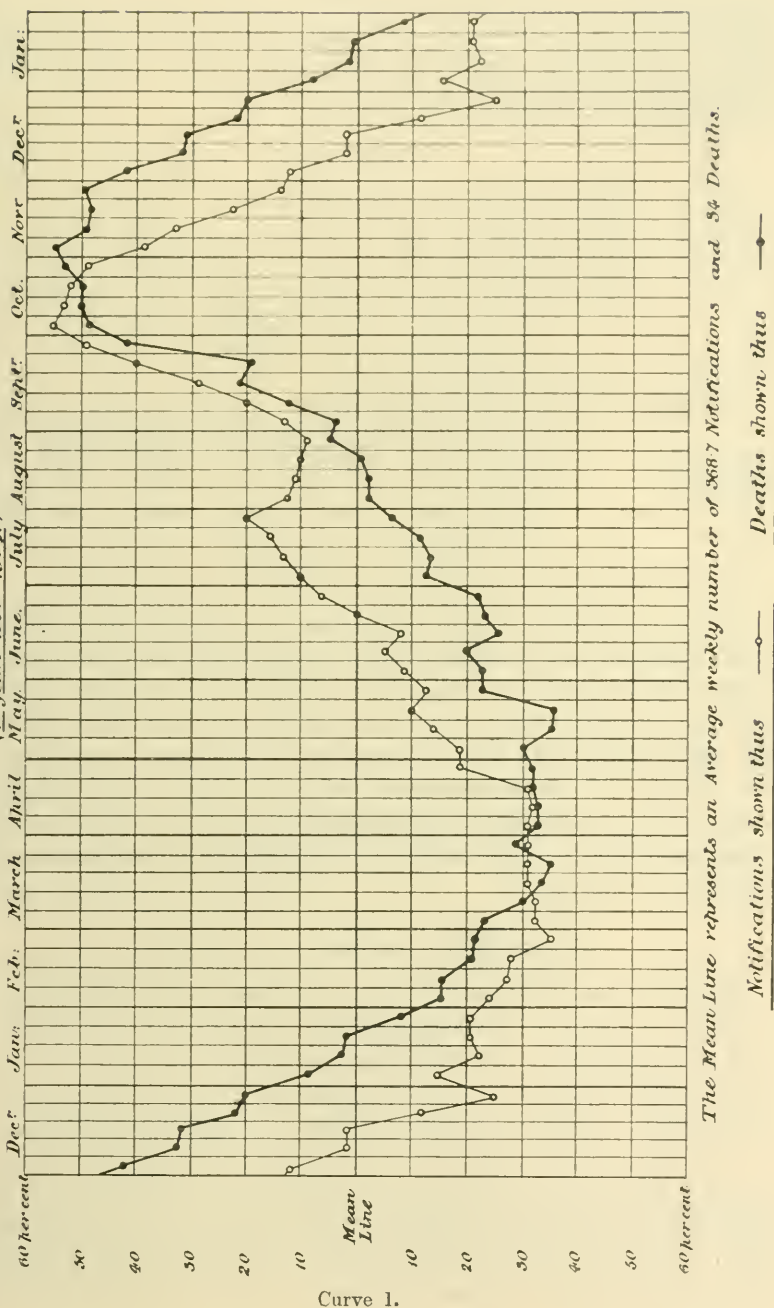
If we accept the hypothesis of a large number of missed cases, then it is fair to assume that the aggregation of children in school should be followed by a rise in the incidence. Such rises or rather variations in the seasonal curve have been shown to coincide with scholastic activities. The contention however arises that they may be dependent on the work of the School Attendance Officers and may really support the belief that there is a large error of omission. The fact that in the accompanying diagram the fatality curve is continuous, whilst the curve for incidence is disturbed in August and January, does suggest that the error of omission is larger at these periods.

Regarding Scarlet Fever as a disease with a short incubation period, we might expect the daily return of cases to be modified by the school closure on Saturday and Sunday;—few notifications are for obvious



Scarlet Fever Notifications and Deaths. (LONDON) Dr. Caiger:

(1.5 years 1881-1904.)



Curve 1.

reasons received as dated on a Sunday. If, however, we divide our returns into those from 0 to 5 years and from 6 to 12 years and regard these as two random samples of a universe, we have two series that are comparable. The figures are as follows:

TABLE IV.

Ages	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Total
0 to 5 yrs.	54	50	59	42	40	55	31	331
Proportion	·1631	·1510	·1782	·1270	·1208	·1662	·0936	
6 to 12 yrs.	119	105	108	97	95	105	66	695
Proportion	·1722	·1511	·1554	·1395	·1367	·1511	·0950	
					Total	...	...	1026
					$X^2 = 1.3144,$		$P = .99989.$	

Brownlee (1910) gives the following:

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Actual number notified	124	143	117	134	120	143	126
Theoretical value	129.6	129.6	129.6	129.6	129.6	129.6	129.6
	$X^2 = 5.18,$		$P = .522.$				

That is half the trials made would give as much divergence as actually found.

From these figures  $X^2 = 1.3144$  and  $P = .99989$ , or the odds are 9 to 10 against the two distributions being dissimilar. This is smaller than the figure found by Brownlee. He also shows (1902 and 1904) that the same deductions can be made when the age incidence, before and after the introduction of compulsory elementary education, was introduced. We can conclude from this that a school distributes Scarlet Fever to approximately the same extent as the home and other outside influences. We are not justified in saying that the schools do not disseminate the disease.

#### *Susceptibility and chance of infection.*

From the point of view of our inquiry, namely the influence of age of parent at birth on susceptibility, it would greatly strengthen conclusions drawn from any bias that the figures may show, if we can assume that all persons of the population are uniformly exposed or that susceptibility is a more important factor than chance of infection.

If we assume the truth of the data already produced, it does seem probable that all are breathing a more or less diluted infection and that the factor which decides whether they will react or not is their susceptibility. To get some idea of the amount of infection that exists in the general population, we must take away a certain proportion and see

what happens to the susceptible units, which are known to exist. This experiment has been done on a large scale owing to the provisions of the Act of 1899. We are able by this means to investigate, firstly the effect of removal on infection in the home, by comparing isolated and non-isolated cases, and secondly the effect on any given area, when the proportion isolated is variable.

TABLE V.

(1) *The effect of removal on the spread in the house.*

Arnold gives the following figures:

	Cases isolated (Hospital)	Cases not isolated (Home)
Total houses supplying cases ... ..	3213	405
Houses supplying more than one case ...	324	135
Number of children left after primary cases	8472 (2.6 %)	777 (1.9 %)
Number of these attacked ... ..	405 (4.8 %)	187 (24.1 %)
Number possibly due to failure of isolation etc.	201 (2.4 %)	124 (16 %)
Number of "return cases" ... ..	254 (3 %)	—

It can be said that the families of cases treated at home are under better medical supervision than those treated at hospital and hence a smaller error of omission may occur. Against this, the mean size of family of those isolated is larger and the chance of spread more likely (2.6 against 1.9). That isolation does however actually prevent the spread is shown by the infection of some of the susceptible units, after the primary case has returned from hospital.

As to whether 3 % really represents the amount of leakage from such institutions is open to some doubt. Newsholme gives the following data collected from Brighton:

*Secondary cases as rate per cent. of population in immediate contact in the tenement.*

	Attack rate per 1000 amongst those living in same family		Total persons in same house	
	Under 20	Over 20	Under 20	Over 20
Isolation carried out	32.7	4.1	28.8	2.8
Not isolated ...	50.2	5.2	46.3	4.3

which brings out the same point. Further it is seen that inclusion of others living in the same house tends to reduce the percentage infected.

The following figures illustrate this point in another way:

TABLE VI.

Day of disease on which patient was removed	Number of cases of Scarlet Fever		
	One only	More than one	Rates
1	7	—	—
2	19	5	3·8
3	26	5	5·2
4	20	3	—
5	11	—	—
6	2	—	—
7	3	—	—
8	2	2	—
9	1	—	—
10	—	—	—
11	2	—	5·1
12	1	—	—
13	1	2	—
14	—	1	—
15	1	—	—
16	1	—	—
17	1	—	—
18	—	1	—

From the run of the figures, delay in removing the primary case tends to increase the number of secondary cases. It is possible that the larger the family, the greater the delay in sending for Medical Assistance, owing to financial and other circumstances (still the conclusion drawn may be safely inferred).

To make these comparisons more complete "return cases" should be included. Although many observers have held that they depend upon what is technically known as "Hospitalism," Newsholme has strongly dissented from this view. The following figures given by Sørensen show a sequence which suggests that a person leaving hospital does bring something with him which slowly diminishes either through time or the exhaustion of the susceptible material by means of which its presence is detected. The figures are as follows:

TABLE VII.

*Interval between discharge of patient and return of another  
from same house.*

2 to 10 days	...	...	...	163
11 ,, 20 ,,	...	...	...	77
21 ,, 30 ,,	...	...	...	30
31 ,, 38 ,,	...	...	...	2
39 ,, 70 ,,	...	...	...	2



On the other hand, Chapin has shown for the City of New York, where it is usual to keep the infected member at home and send the others away—a method contrary to ours, that out of 317 healthy children sent away, eighteen were attacked on their return, being a percentage of six. Arnold states that out of 8472 children remaining at home amongst whom 3213 were received from hospital, 254 were infected, or a percentage of 3.0. This would seem to negative the idea that patients collect infection in their systems whilst in a hospital ward, hence we are justified in grouping Neech's material in the following manner.

The figures are taken from the experience of Halifax, and relate to the length of time taken by a family to react to the introduction of infection and the influence of hospital isolation. They are of interest in showing to what extent infection may be present after considerable lapse of time and that the periods given must necessarily be the minimum as the susceptible material by means of which infection is detected soon becomes exhausted.

TABLE VIII.

	Years	Primary cases	Secondary cases after interval of										
			1 week to 6	7	8	9	10	11	12	13	14		
No hospital	1887	1169	184=97 %	2	1	1	0	0	0	1	1		
	1888											6=3 %	
Hospital	1901	1188	110=62 %	7	2	9	9	15	11	4	9		
	1902											66=37.4 %	

The question is, to what extent after an attack of Scarlet Fever does infection persist in the person attacked? To elucidate this problem we have taken the initial case away for a period of time, and then compared the spread of the disease in the family, with a period in all respects apparently similar, except that the initial case was not removed.

In Arnold's data already given, we have seen that the comparison of families in which cases were and were not isolated is not applicable, as the families are essentially different in social status, and in the medical supervision received. This does not apply to Neech's data and if we can assume that in Halifax the type of disease was similar in the years cited, the material is strictly comparable. Taking the first six weeks of the 1887 to 1888 period and adding to the corresponding weeks of

the 1901 to 1902 period the cases that occurred from the 7th to the 12th weeks we have:

	Initial cases	Secondary cases					
		1st week	2nd week	3rd week	4th week	5th week	6th week
1887   1888	1167	75	60	24	16	4	5
		1st and 7th week	2nd and 8th week	3rd and 9th week	4th and 10th week	5th and 11th week	6th and 12th week
1901   1902	1188	71	24	23	16	17	12

The question is: what is the probability of these two series being samples of different universes?

$$X^2 = 26.675 \quad \text{and} \quad P = .017.$$

Hence the odds are about 50 to 1 against the distribution being taken from the same material. It must be borne in mind that it was assumed that all cases were discharged at the 6th week, which is not correct and it is probable that had we been able to place "return cases" in their appropriate weeks from the date the infecting case left hospital, some of the cases placed in the 5th and 11th weeks and 6th and 12th weeks would have found their way to the 2nd and 8th weeks, the fit being improved; still from the data already given it is justifiable to say firstly that removal of the primary case delays secondary infection and secondly reduces the number infected in the home, the actual reduction being probably small.

(2) *The effect of isolating different proportions on general prevalence.*

(a) *Different periods of same district.*

We have a useful series given by Dr Walford for the City of Bristol, though the difficulty in drawing a conclusion is that the type of disease varies from year to year. The figures are in the order of the percentage isolated.

TABLE IX.

Years	Attack rate 15 and under	Percentage isolated
1891	13.4	—
	32.1	13
↑	14.9	22
	10.3	31
	8.5	43
	21.4	47
to	21.7	48
	14.8	48
	13.1	50
↓	5.3	56
	14.1	63
	6.1	65
1903	2.7	66

From this it will be seen that the greater the number isolated the smaller the attack rate. The association may however be spurious as the disease itself is a very variable factor.

The following series of data bear out the point in more detail. They are taken from the Metropolitan Asylums Board's records given by Parsons in a report on Isolation Hospitals to the Local Government Board 1910-1911, page 8.

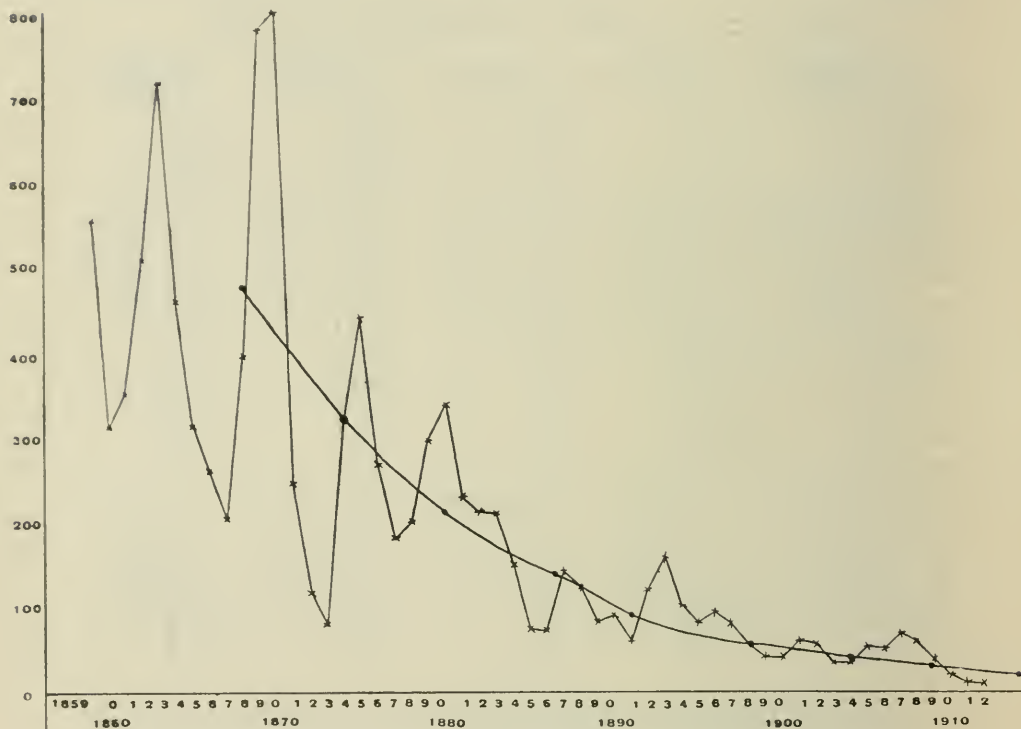
TABLE X.

*Scarlet Fever in London.* (H. Franklin Parsons, M.D.)

Year	Notifications per 1000 population	Deaths per 1000 population	Case Mortality per cent.	Percentage of cases removed to Hospital	Case Mortality per cent. in Hospitals
1890	3.7	.21	5.6	42.8	7.9
1891	2.7	.14	5.1	46.8	6.7
1892	6.4	.27	4.3	48.8	7.3
1893	8.6	.37	4.3	39.7	6.1
1894	4.3	.22	5.2	63.9	5.9
1895	4.5	.19	4.2	58.2	5.4
1896	5.7	.21	3.7	62.6	4.3
1897	5.1	.18	3.4	67.0	4.1
1898	3.8	.13	3.4	73.2	4.1
1899	4.1	.09	2.2	74.3	2.6
1900	3.1	.08	2.6	75.1	3.0
1901	4.1	.13	3.2	78.9	3.8
1902	3.9	.12	3.1	80.3	3.4
1903	2.8	.08	2.9	83.8	3.1
1904	3.0	.08	2.7	84.5	3.4
1905	4.3	.12	2.8	88.6	3.3
1906	4.4	.12	2.6	88.5	2.9
1907	5.8	.14	2.5	89.4	2.8
1908	4.8	.12	2.5	90.9	2.6
1909	3.8	.08	2.2	90.2	2.3
1910	2.5	.05	2.0	85.6	2.3

From these figures it is seen that the increase in the proportion of cases admitted to the Isolation Hospitals is associated with a fall in the number notified per 1000 population, in the death rate and in the case mortality. The rates are based on large numbers and can be taken as significant. Some doubt, however, is thrown upon the nature of the association when we consider the history of the Scarletinal death rate, previous to 1890, the year in which comprehensive Isolation of Scarlet Fever can be said to have begun in the Metropolitan Area. The following diagram is taken from a paper published by Dr Brownlee (1913) dealing with the periodicity of Infectious Diseases.

A curve has been fitted to the variations in the Scarletinal death rates from 1868 onwards, from which Dr Brownlee remarks, "The irregular curve of the epidemic waves, swings very evenly above and below the curve of the geometrical progression." "If the peak in 1875 is taken as the starting point it may be noticed that it is followed by a lower but broader epidemic wave culminating in 1880. The same phenomenon is repeated on a lower level beginning in 1887, and again in 1901. There is thus a complex fourteen year cycle in the epidemic wave."



Curve 2.

It will be noted that there is nothing in this curve which in any way demarcates the difference in the practice in the treatment of the disease in the neighbourhood of 1890. It would be of interest, therefore, to correlate the attack rate with the percentage isolated, the case mortality being kept constant. That is to say, what would the effect of isolation have been had the severity of the disease remained constant?



The most useful series of figures that I have been able to find bearing on this point are those given in the Annual Reports for the City of Nottingham, in which we have the case rate per 1000 population, the percentage removed to hospital and the case mortality for the years 1882 to 1910. The Nottingham series are of especial value, for from the year around about 1894 the tendency has been for the number isolated to diminish. It would be, on this account, reasonable to expect some increase in the disease in the later years. The series includes three years, 1882-1884, which were prior to the establishment of a hospital.

The table is as follows:

TABLE XI.

*Scarlet Fever in Nottingham.* (P. Boobyer, M.D.)

Year	Cases	Case rate per 1000 population	Death rate per 1000 population	Case Mor- tality per 100 cases	Percentage removed to hospital	Cases per household invaded	Return cases per 100 dis- charged from hospital
1882	1029	5.2	1.45	27.8	—	1.15	—
1883	428	2.2	.29	13.8	—	1.2	—
1884	384	1.8	.18	9.6	—	1.12	—
1885	390	1.8	.14	7.7	12	1.2	—
1886	351	1.6	.06	3.7	15	1.4	—
1887	615	2.9	.10	3.6	45	1.4	—
1888	643	3.0	.11	3.9	49	1.3	—
1889	1047	4.8	.13	3.2	71	1.2	—
1890	984	4.7	.15	3.3	81	1.3	—
1891	895	4.2	.13	3.2	86	1.39	—
1892	1163	5.4	.19	3.7	88	1.13	4.0
1893	1511	6.8	.37	5.4	70	1.2	5.0
1894	1164	5.3	.23	4.2	80	1.17	4.5
1895	1250	5.5	.23	4.1	93	1.3	5.1
1896	731	3.2	.11	3.7	86	1.4	2.3
1897	517	2.2	.15	6.6	90	1.2	3.0
1898	931	3.9	.14	3.5	71	1.5	3.4
1899	2500	10.8	.23	2.0	47	1.2	1.5
1900	1394	5.8	.23	4.0	49	1.2	3.2
1901	918	3.8	.05	1.2	47	1.1	5.5
1902	966	4.0	.10	2.1	52	1.1	3.68
1903	1420	5.8	.14	2.4	34	1.2	2.18
1904	1189	4.8	.11	2.3	39	1.2	3.48
1905	681	2.7	.07	2.8	56	1.35	1.28
1906	611	2.4	.07	2.8	57	1.26	1.17
1907	416	1.6	.02	1.2	69	1.2	1.76
1908	595	2.3	.04	1.85	52	1.1	2.27
1909	1036	3.9	.04	.96	54	1.1	2.56
1910	697	2.6	.06	2.0	60	1.3	2.6

Correlating these variables, the percentage number of cases isolated (1) and the attack rate per 1000 population (2),  $r_{12} = \cdot 1024 \pm \cdot 1336$ .

The percentage number isolated (1) and fatality rate (3)

$$r_{13} = - \cdot 2903 \pm \cdot 1228.$$

*Note.* If the years 1882-1884 where no cases were isolated be ignored, then

$$r_{13} = - \cdot 0031 \pm \cdot 1349.$$

Case rate per 1000 population (2) and fatality rate per 100 attacked (3)

$$r_{23} = - \cdot 3064 \pm \cdot 1228.$$

From these figures it might be assumed that a decrease in the number isolated has been followed by the decrease in attack rate or in other words it might be said that the town has participated in the general fall of Scarlet Fever irrespective of hospital isolation. If we now make the case death rate constant then the correlation between the attack rate and the percentage removed will be  $r_{12} = \cdot 0147 \pm \cdot 1340$ .

Hence there is no legitimate evidence in these figures to believe that the practice of isolation has reduced the amount of floating infection in the community under consideration.

(b) *Towns isolating different proportions.*

If we assume that isolation affects the spread in the home and does not influence appreciably the amount of floating contagion in the community generally, it is possible to calculate, on the basis of the Croydon experience, what the rate would be in any group of towns isolating a varying proportion, supposing all were removed to hospital. A useful series has been collected by Dr Kaye and is as follows:

TABLE XII.

	Isolating less than 20 %	Isolating between 20 and 50 %	Isolating between 50 and 80 %	Isolating over 80 %
Number of towns in each group	25	42	17	9
Gross population ... ..	1,513,347	5,346,808	2,222,000	1,036,683
Total no. of cases of Scarlet Fever	7,037	22,831	9,235	3,815
Cases isolated ... ..	1,407	7,991	4,156	3,052
From the above we have:				
Cases not isolated ... ..	5,603	14,840	5,079	963
And on the basis of the Croydon figures the numbers of second- ary cases will be ... ..	1,778	4,688	1,604	304
If all had been isolated the number of secondary cases would have been ... ..	789	2,082	712	135
Thus the total number of cases would have been if all had been isolated ... ..	5,048	20,225	8,343	3,646
Giving a rate of ... ..	3.34	3.78	3.76	3.51
The actual rate was ... ..	4.65	4.27	4.16	3.68

Thus the apparent effect of isolation disappears, an inference that Millard had already made when towns like Preston and Warrington were compared. To solve this problem, it should be handled in the same manner as has been already used in elucidating the influence of overcrowding, size of family, etc. As this paper does not concern itself with the utility of the isolation hospital, the rather laborious task of handling a problem with at least six variables has not been undertaken<sup>1</sup>. It can be concluded that the effect of removing cases from the home, even in spite of leakage from the hospital, through "return cases," has more effect on the production of secondary cases in the home than removal of all known cases has on the prevalence of the disease in the community. Hence there must be some other source from which infection arises, and it must be such as is hardly influenced by the isolation of known cases. This may arise through

- (1) A large number being missed.
- (2) A large proportion reacting atypically.
- (3) Infection persisting in certain cases for a considerable period of time.
- (4) Infection arising through other channels than personal contact.

Evidence of the large error of omission has been already given. That the second cause is an operation factor is also probable. Jürgensen, Johannessen and Thomas emphasise the fact that Angina directly consequent on Scarlet Fever need not be accompanied by a rash. Further, in various epidemics, instances of which are given in the literature quoted, outbreaks have either been preceded, or accompanied by a number of cases of sore throat. It has been observed in the Barking School Clinic that the autumnal rise is preceded by an increase in the number of throat ailments sent to the clinic from the schools for diagnosis and advice. The figures are as follows:

	Jan.—July	Aug.	Sept.	Oct.	Nov.	Dec.
Cases of Scarlet Fever ... ..	61	4	18	29	28	22
Cases of Angina and Tonsilitis ...	10	3	21	6	5	3

The same sequence has been observed each year, so that the figures cannot be said to be a chance happening.

<sup>1</sup> K. Pearson and E. Elderton in a paper on the Influence of Isolation on the Diphtheria attack and death rate (*Biometrika*, x. 1915. p. 549) have come to a similar conclusion, details of which are given later in the paper.

The following data, given by Butler, lend support to this view, and relate to the existence of sore throats previous to the occurrence of a definite case of Scarlet Fever in an individual house.

	Scarlet Fever	Other diseases
No. of houses infected ... ..	1266	1644
History of previous sore throats	395	47
Percentage ... ..	31.2	2.8

It is stated that adults were more frequently attacked, but such conditions in children are more likely to be overlooked.

In connection with the third factor concerned in the failure of isolation to modify the prevalence of the disease, it is of interest to examine whether the usual figure of 3-4 % of infecting cases (that is, cases which are definitely known to infect others in the same household after isolation), really represents the number that leave the hospital in that state.

The following figures show that the persistence of infection depends on the severity of the attack. They are taken from Sørensen's observations on Scarlet Fever in Copenhagen.

TABLE XIII.

Year	No. of cases isolated	Attack rate per 1000 population	Death rate	No. of return cases per 1000 discharged		
				Less than 10 days	Over 10 days	
1893	1,758	9.9	3.3	—	—	3.8
1894	2,137	16.4	4.2	—	—	3.1
1895	1,050	5.5	3.8	1.9	2.7	3.3
1896	781	4.5	2.3	2.0	3.7	4.0
1897	676	4.2	1.4	2.2	2.5	3.6
1898	913	5.9	2.2	1.8	2.7	2.4
1899	1,763	10.9	2.5	2.5	3.4	3.9
1900	1,408	6.5	2.4	2.7	3.8	4.5
1901	813	3.3	2.4	1.9	3.5	4.1
Total	11,299	3.6	—	—	—	—

Considering the same point with respect to the nature of the population to which cases discharged from hospital are sent, we have the following data taken from Barking. One hundred and ninety-eight cases discharged from hospital were followed by the recurrence of the disease in other members of the family in ten instances within one month. The age distributions of the families were as follows:



TABLE XIV.

Age	Number	Number said to have been previously attacked
No children at home	21	—
Under 1 year	26	—
1-2	15	—
2	28	—
3	27	—
4	24	1
5	33	—
6	28	—
7	32	2
8	23	3
9	28	4
10	22	4
11	19	1
12	23	1
13	26	4
14	20	3
15	22	2
16	11	1
17 and over	12	—
	440	26

Hence the attack rate is 10 in 440 or 22 per thousand in one month. The rate for the population 16 years and under in Barking, for a corresponding period, was 3·2 per 1000.

It is of interest to note that the attack rate based on "return cases" amongst such a population is seven times as great as that for the whole town. The actual number of cases leaving the hospital in an infective state must be fairly high, for it is unlikely that in every instance "return cases" will follow in the family to which an infective case is discharged.

Now it was found that during the same period 15 % of the above notifications were secondary cases, and if we assume that secondary cases are due to the primary one, then a large proportion (85 %) of the families which supplied one case only must be relatively insusceptible. Hence such a family when it again receives its infected member is not likely to furnish a return case. Should we assume that such cases as are discharged from hospital have the same infective power as "initial" cases, then the number leaving hospital in that state must be  $\frac{85}{15} \times 5 = 28$  %. This would seem to indicate that every fourth child leaving hospital irrespective of discharges from nose or throat is capable of causing further cases even after six weeks isolation. Support is lent to this view that infection is very persistent in a large

proportion of cases, by the frequently observed outbreaks of Scarlet Fever, after operation for chronic suppuration of the middle-ear amongst the inmates of a childrens' ward, even when the attack of Scarlet Fever upon which the condition was dependent had occurred many years before. Boeck gives an instance where the infection was apparently transmitted through the medium of hair, after twenty years, and Elliston, quoted by Murchison, gives instances of personal infection after two years.

The period of six weeks must be looked upon as a purely arbitrary standard and is based more on the demands of the general public than on the reasoned advice of the medical profession. The last of the series, namely whether infection may arise from other sources than personal contact, requires some attention, though the facts already given are sufficient to account for the endemic nature of the disease.

*Milk as a possible source of infection.*

Scarlet Fever has been associated with almost all articles of diet, though milk only has supplied sufficient evidence to make it worthy of consideration.

Up to 1885, milk was supposed to convey the infection in a passive manner, but Klein and others have thought that a micrococcus, transmitted from the cow, was the specific cause<sup>1</sup>. The active theory of the agency of milk has not received confirmation from later observers, so that the original position has been reverted to.

Kober collected instances of 330 milk epidemics of which 243 occurred in England, 52 in America, 11 in Sweden, 10 in France, 2 in Germany and 12 in other countries, and he suggests that in England and Sweden much milk is consumed in a raw state and might explain the differences in the proportion noted. Hall also shows that in Japan and China and India, Scarlet Fever is unknown and milk is not consumed. Neither of the statements is really accurate, for Ashmead has described a disease as Scarlet Fever in Japan which if not common is certainly distinct, and closely resembles Scarlet Fever.

Kober also shows that even if an undue proportion of cases fall upon a certain milk supply, the cause is not certain, as it is quite common for certain supplies to run in certain parts of a community where other

<sup>1</sup> The outbreak at Hendon in 1885 investigated by Klein and Power (see Rep. Med. Off. Local Gov. Board 1886-7, Klein, *Proc. Roy. Soc.* Vol. XLIII.) is well known in this connection.

means of contact are quite common. Hamilton after a close study of all available literature divides milk epidemics into two classes.

(1) The larger, which includes those which must be ignored, as the statistical evidence is not adequate, a class which arose from the erroneous belief that the cow suffered from a disease communicable to man.

(2) The smaller group, which present evidence which she regards as above suspicion, and can be attributed to the fact that under certain circumstances milk offers a suitable medium for the propagation of the virus.

Freeman gives the following as characters of a milk epidemic.

(1) *The disease appears suddenly and subsides equally suddenly.*

The actual occurrence of cases has been examined by Brownlee and he finds, in the case of an epidemic in Glasgow, that a "normal" curve gives a very good fit. The seasonal rise and fall also can be fitted in a similar way, so that the difference between a milk epidemic and ordinary incidence is one of degree and not of kind.

A group of cases due to milk infection may however be the beginning of an epidemic dependent on personal contact. The following figures are taken from an epidemic at Weymouth in 1901 where the first few cases appear to have been due to milk, though subsequent contact appears to have been responsible for the extensive spread. It is compared with the epidemic at Thorshavn in 1873, where the origin was from one case only. Weymouth had been fairly free from Scarlet Fever, whilst in Thorshavn no case had been observed during the generation then alive.

	Population	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May
Weymouth	15,400	—	21	14	52	36	16	6	—
Thorshavn	930	4	118	72	21	5	12	2	2

It is seen that in the latter the rise is sudden, more so than in the former. Other examples could be given bearing on the same point. It would appear that the steepness of the curve may depend on the nature or distribution of the population rather than on the source of the infection. The curves of different epidemics are so variable that it would be hardly safe to draw any conclusion from the peculiarities of any particular one.

(2) *The houses invaded are quite distinct and not restricted.*

Generally speaking it is the exception, rather than the rule, to be able to trace the origin of infection in primary cases. This can hardly be a character of a milk epidemic.

(3) *The houses invaded belong to the rich rather than the poor.*

This is no peculiarity of a milk epidemic. The ubiquitous character of Scarlet Fever is noteworthy and, as has been shown, its diagnosis may be a measure of the ability of the family to procure medical assistance.

(4) *The incidence of a milk epidemic. It is said that 78 % of those attacked are milk drinkers, whilst in ordinary epidemics the figure is only 27 %.*

This is not a useful criterion, as the milk supply even of a small village is usually from several sources, and it does not take into account the alternatives of an epidemic being started by milk and spread by personal contact, or being wholly due to milk infection. It is plausible to suppose that in the latter case it should partake of the character of an epidemic of meat poisoning, such as occurred at Chesterfield, Derby and elsewhere. In any case such estimates are of little help in a decision.

(5) *It has been suggested that multiple cases are more frequent in milk epidemics than in endemic incidence.*

The following figures lend no support to this contention.

	Multiple cases (Weymouth) Milk epidemic, 1901 (Sept.—Jan.)	Multiple cases (Middlesbrough), 1910 (Sept.—Jan.)
Houses not invaded	4290	20,165
With 1 case	80	65
.. 2 cases	15	20
.. 3 „	5	9
.. 4 „	3	3
.. 5 „	5	—
Totals	108	97

There is nothing very different in these two series. In the latter the incidence is less, and the proportion of multiple cases is greater. If we compare the Autumn and Spring incidence in any town it is seen that multiple cases are more frequent in the latter than the former period. It would thus appear that they depend on the virulence of the infection rather than the agent by which it is spread.

(6) *It has also been thought that Milk Scarlet Fever is more prone to attack adults than children.*

Comparing the classic epidemic at Thorshavn and the milk epidemic at Weymouth, we see that age of attack is a factor dependent on the previous history of the disease. In any community which has been free for a considerable period, the incidence tends to be on adults.



TABLE XV.

*Thorshavn.*

Age	No. living	No. attacked	Rate	Milk epidemic (Weymouth)	
				Age	Rate
0-1	12	8	66·6	0-5	2·4
1-5	65	44	67·1	5-10	5·3
5-15	155	99	63·9	10-15	3·2
15-20	56	42	75·0	15-20	1·6
20-40	171	40	23·4	20-30	2·1
40-60	105	3	2·9	over 30	1·4
over 60	55	1	1·8		3·5

It is possible that more information would be obtained were care taken to separate primary cases, that is, the first case occurring in any household should only be considered. In no epidemic of any considerable size, as far as my search has gone, have I been able to separate the cases as described. With the information given in this manner, the only real statistical test can be applied and the chance calculated of an undue proportion falling to any particular milk supply. In the town of Barking there are twelve wholesale supplies. The problem is, what is the chance of 50 % or more of a series of 100 consecutive cases of Scarlet Fever falling to one milk vendor? By calculation it was found that this should happen about once in 25 years. In so far as approximately 100 cases are notified annually, and as such a grouping has not occurred in the last 25 years, though search has been made, milk cannot be said to be a cause of the disease in this district. Support is lent to this deduction by the following data.

Inquiry was made of 400 households, which contained a child of school age. This age period was chosen because such a sample would be a fair reflection of the households from which a case of Scarlet Fever was likely to occur; the mean age of attack of Scarlet Fever being a little over seven years. Comparing this with the source of the milk supply of these households where Scarlet Fever occurred we have:

Random samples	Condensed milk and no milk	Fresh milk				
		A	B	C	D	E
357	92	31	23	46	48	117
Scarlet Fever cases						
320	87	37	31	39	49	77

Classes A to D are definite, class E includes those where the source was indefinite or where the supply came from two or more dealers: it also includes those which had less than 20 customers.

From this

$$X^2=12\cdot8 \text{ and } P=\cdot29.$$

If we assume that condensed milk cannot convey infection (I have only come across one instance of a small outbreak of Scarlet Fever which was attributed to condensed milk and in that instance the evidence was very meagre), we see that those who take no milk are just as likely to be attacked as those who consume fresh milk, if anything, rather more so. Before any milk supply is blamed, some figures such as the above should be collected and the chance evaluated of any variation in the distributions being significant. In so far as the milk supply in this country is very carefully watched, some of the distributions reported may be due to chance variation, and as in all I have only been able to get references to fifty-seven, we should hardly be justified in saying that milk is a frequent source of infection. In thirty-seven of the epidemics in which the evidence seems good there was contact with a previous case, either at the milking or shortly afterwards. This seems to suggest that the time when milk will absorb the infection is from time of milking to a few hours afterwards, that is, whilst the milk is warm and comparatively sterile. Instances of contamination of milk with Scarlet Fever infection in retail shops with no traceable cases is a frequent occurrence in any district. It would thus appear that when milk is cold and the lactic fermentation well advanced, it is apparently not a suitable medium. I am unable to trace any instances of butter or butter milk being the source of infection, though the processes to which it is subjected should render it liable to contamination.

From the evidence that has been gathered we can conclude firstly, that the main factors concerned in the maintenance of the disease are (a) atypical cases, (b) missed cases, (c) persistence of infection (carriers) in a certain proportion, and secondly, that in so far as isolation of known cases does not materially influence the chance of infection outside the home, the amount of floating infection must be extremely large. All must in the ordinary course of events come into frequent, almost daily contact with one or other of the above classes. Hence we must assume that susceptibility is a much greater factor in reaction to the presence of this parasite, than chance of infection.

The next point that requires consideration, in relation to errors of excess and defect, is to what extent do these categories differ in age distribution from a population composed of cases of true Scarlet Fever. The mean ages for the group considered under errors of excess should be the greater, for a case returned erroneously might be selected from any age period.

The following figures bear this out:

*Cases returned as Scarlet Fever and found to be suffering from other diseases. (Barking Town Isolation Hospital, 1913-1914.)*

	0-5 years	6-10 years	11-15 years	16 and over	Mean age years
Not Scarlet Fever cases ...	8	9	6	3	9
Scarlet Fever cases ...	36	178		11	7.4
General population per 1000	136	128	120	636	—

It is obvious that errors of excess cannot be regarded as a random sample of the general population, nor do they appear to coincide with a true Scarlet Fever distribution. The numbers are too small to allow one to examine the point in any detail.

No data are obtainable with respect to the effect of the inclusion of "errors of defect" on the mean age of attack. It seems likely, however, that the period during which any disturbance in health is likely to be overlooked, is from the 7th to the 14th year, that is during this period when the child is changing from a dependent to an independent position. The point is of some importance in our subsequent inquiry and it is to be regretted that a more definite statement cannot be made.

It may be questioned as to whether data subject to such large errors be worthy of detailed examination, but as some questions of great interest have arisen, their analysis may not be without profit.

Before discussing the main theme it will be advisable to examine in what way the age curve of incidence is modified by susceptibility.

#### *Age incidence and susceptibility.*

To elucidate this, a group of children were obtained during the course of medical inspection, the selective factor being the presence of such parents as thought fit to attend the examination, and the point as to whether an inherited predisposition to the disease existed was examined. An ordinary fourfold table was formed, the figures being as follows:

	Children		Totals
	Not had Scarlet Fever	Had Scarlet Fever	
Mothers not had Scarlet Fever	1042	388	1430
„ had Scarlet Fever ...	140	94	234
Totals	1182	482	1664

$$r_1 = .152 \pm .0286.$$

On the assumption that the above figures are diluted, as far as the children are concerned, with 50 % of uncorrelated data, then (notation as on p. 212)

$$\frac{r_1}{r_2} = \frac{n_1}{n_1 + n_2},$$

$$\therefore r_2 = \cdot 304.$$

This allows only for errors relative to children. It does not account for defects of memory, or ignorance with respect to parents. From this it can be safely assumed that there is a strong inherited predisposition not necessarily to the invasion of the parasite but certainly in reacting to its presence by the production of the symptoms which give the disease its name.

If heredity plays a part in susceptibility, we should expect certain families to be more frequently invaded on distinct and separate occasions than chance would lead us to anticipate.

The following figures given by Dr Butler with respect to the house are consistent with such a view. The approximation after a five years' interval may be due to the increasing number of removals, the house being no longer synonymous with the family.

Second notifications received from same address after varying intervals.

TABLE XVI.

Interval	Expected	Actual
3 weeks-3 mths	1.4	20.25
3 mths-6 "	1.7	6.75
6 " -12 "	3.5	6.1
1 yr -2 yrs	6.6	10.0
2 " -3 "	5.7	8.0
3 " -4 "	6.2	6.6
4 " -5 "	6.4	7.0

It is of course probable that the large percentage in the first three periods given is due to infection in the family itself. From the 12th month onward, the cause suggested above may be an operative factor, and in so far as some families will be entering and others leaving the danger zone, the excess of the actual over the expected should decrease as the interval gets longer. That heredity however is concerned in the manner in which the organism reacts to the presence of infection is borne out by Thomas (Ziemssen's *Handbuch*), Copeman, Harlin quoted by Trouseau, Johannessen and Seitz—all of whom cite instances



of variations in the characteristic features of the disease peculiar to a particular family.

Hence we are justified in grouping our children into those who have, and those who have not, a history of the complaint, and comparing the age distribution of attack.

From this we should get some idea as to the way in which susceptibility alters the curve of age incidence. The following figures were obtained:

TABLE XVII.

Age of child when attacked	Mothers who have had Scarlet Fever	Mothers who have not had Scarlet Fever			
0	2	0			
1	1	11			
2	16	13			
3	11	19			
4	13	35			
5	18	40			
6	17	44			
7	13	36			
8	13	16			
9	6	10			
10	0	15			
11	6	10			
12	6	6			
13	—	9			
14	1	5			
15	2	3			
16	2	2			
17	—	—			
18	1	1			
19	4	—			
20	2	—			
			Number	Mean age	Standard deviation
Children of mothers said to have had Scarlet Fever			134	6.7686 $\pm$ .1508	2.8195 $\pm$ .1161
Children of mothers said not to have had Scarlet Fever			275	6.8509 $\pm$ .0729	2.5356 $\pm$ .0729

It is seen from this that the mean age of attack is not altered, but that in the case of children of mothers who have not had the disease the scatter is less but the difference is hardly significant. Hence we may conclude that susceptibility does not alter the position of the axes of the curve of age incidence.

*Age of parent at birth and age of attack.*

We can now turn to the actual data with some hope of interpreting any trend they may show. The figures were obtained from the records of the County Borough of Middlesbrough 1904-1912 of such cases as were notified to the Health Department as suffering from Scarlet and other Fevers.

Close on 3000 were notified during this period and 1991 afforded the information required. The following points are to be noted.

- (1) Only such cases where the mother was alive were taken.
- (2) All over nineteen were ignored, as it was found that even before this age the person attacked was not living in the same house as the parents and hence could not be included.
- (3) No correction is made for errors of diagnosis.

TABLE XVIII.

*Age of mother at birth of offspring and age of attack of Scarlet Fever. All parents living.*

Age of mother at birth of offspring															Totals
Age of attack	18-19	20-21	22-23	24-25	26-27	28-29	30-31	32-33	34-35	36-37	38-39	40-41	42 and over		
0	—	2	0	3	2	4	1	0	2	0	1	2	1	18	
1	—	4	6	7	6	8	4	6	4	3	2	2	—	52	
2	—	7	7	31	23	14	13	10	6	7	6	3	2	129	
3	2	11	23	30	31	27	19	8	6	5	10	3	3	178	
4	—	11	27	33	42	35	23	17	15	10	6	9	7	235	
5	3	28	27	34	41	42	25	20	18	8	12	4	6	268	
6	2	18	34	37	23	25	25	22	6	7	4	6	4	213	
7	1	19	30	17	21	33	19	10	9	7	6	6	10	188	
8	2	12	12	22	20	19	16	10	10	8	6	6	4	147	
9	5	18	16	18	24	9	13	8	7	5	5	3	2	133	
10	1	10	13	18	14	14	11	7	12	4	3	5	4	116	
11	2	12	9	12	11	13	12	2	8	4	2	4	1	92	
12	—	8	7	6	8	5	2	7	2	1	4	1	2	53	
13	—	6	3	3	4	1	1	1	2	1	0	0	3	25	
14	—	3	9	9	7	4	2	0	4	—	—	—	—	38	
15	—	4	3	1	6	3	3	2	2	6	1	1	1	33	
16 to 20	2	4	4	15	7	14	9	3	6	3	2	1	3	73	
Totals	20	177	230	296	290	270	198	133	119	79	70	56	53	1991	

$$\sigma_{\text{age of attack}} = 3.38,$$

$$\sigma_{\text{age at birth}} = 2.96,$$

$$r = .0206 \pm .0142$$

The coefficients found are:

Age of mother at birth of child and age of attack	...	...	...	$r = .0206 \pm .014$
Correlation ratio of means of age of attack for arrays of mother's age	...	...	...	$-.0966 \pm .012$
Corrected	...	...	...	$-.06$
Correlation ratio of means of mother's age for arrays of child's age	...	...	...	$-.1080 \pm .011$
Corrected	...	...	...	$-.04$
Coefficient of contingency	...	...	...	$-.2510 \pm .010$

The distribution, therefore, is not a random sample, though it does not indicate that a significant and continuous increase in age of attack accompanies a corresponding increase in age at birth. There is a suggestion that the regression line is non-linear and that the mean age of attack is lowest between the 24th and 25th year of the mother. This is more easily appreciated in the following series of figures.

Dividing the data into groups of approximately 200, the means are:

Age of mother at birth	Number	Age of child at attack
21 yrs and under	197	7.92 yrs $\pm .14$
22-23 yrs	230	7.37 „ $\pm .13$
24-25 „	296	7.07 „ $\pm .12$
26-27 „	290	6.99 „ $\pm .12$
28-29 „	270	7.00 „ $\pm .12$
30-31 „	198	7.21 „ $\pm .14$
32-35 „	252	7.38 „ $\pm .13$
36 and over	258	7.27 „ $\pm .13$

It has been shown, however, that owing, 1st, to the omission of children of parents who have died, and 2nd, to the fact that infection in the home is more likely to occur in large than in small families and hence those born late are likely to be attacked at a more immature age—that a negative correlation was to be expected. Hence, in a truly representative population the suggestion is that those born late in life are likely to be attacked at the more mature ages.

The fact that those born around the 25th year are attacked earlier than those at either extreme, may be explained by the fact that smaller families are more carefully watched than large, and a smaller number of cases missed, with the result that the mean age of attack falls.

It must necessarily be so if we assume that the error is greater from the 7th to the 14th year than from 0 to 7 years.

In view of the extremely complex nature of the problem, any conclusion must be drawn with extreme hesitation, still as alteration in susceptibility does not necessarily alter the mean age of attack, a slight increase in the mean age may be a reflection of a marked variation in the biological character of the group in question.

## DIPHtheria.

The attempt made to estimate the amount of infection existing in a population at any time is not necessary in the case of Diphtheria, as the actual agent causing the disease can be isolated fairly easily. The difficulty however arises, that in many instances virulent bacilli can be found in the throat of contacts, who do not react, or only very slightly. It is probable that, should a comprehensive survey of a population be made, the numbers classed as free—infection only—slight reaction, etc. would form some type of curve, and the question arises, which is of importance to this statement, at what point should clinical Diphtheria be said to begin. It is obvious, therefore, that clinical and bacteriological Diphtheria will be very different. There is no doubt, moreover, that the organism is as widely spread as the infection of Scarlet Fever and hence all must at one time or another come in contact with infection. What the error of omission is, will obviously depend on what point is taken on the reaction curve, beyond which clinical Diphtheria begins. The error of commission has been already discussed and is larger than that of Scarlet Fever. It would therefore appear that the present observations are much more vague than those already discussed, and as no attempt to purify the returns has been undertaken, the point under investigation is the influence of age at birth on the age at which a reaction of a diphtheritic appearance occurs.

There is, however, one other relevant source of error, namely, the number of instances that were ignored owing to the mother having died. The same calculations will apply as with Scarlet Fever, that is, a negative correlation of about  $\cdot 016$  is to be expected.

Elderton and Pearson (1915), in a recent paper, have investigated the relationship between isolation and attack rates. The material was supplied by E. H. Snell from the records of the City of Coventry and other areas. They find the crude correlation between isolation rate and attack rate for years 1904-08,  $r = \cdot 427 \pm \cdot 063$  and for the four years 1908-12,  $r = \cdot 290 \pm \cdot 069$ .

They show for the two periods that there is no significant relationship between the death rate from Diphtheria and isolation rate and that there is a significant association between the case mortality and attack rate and between case mortality and isolation rate; the figures are  $r = -\cdot 509 \pm \cdot 057$  and  $r = -\cdot 507 \pm \cdot 056$  respectively.



The influence of density of population and prosperity as judged by proportions of domestic servants are considered, and values are found which suggest that the latter is associated with an increased rate and that the former does not appear to have any influence. They draw the following conclusions. "They contain nothing to support the theory that isolation markedly limits the incidence of Diphtheria. The disease itself does not appear where overcrowding is greatest nor where the population is most dense; on the other hand isolation is most practised in those towns where domestic servants are most common and which may be supposed to be most prosperous. The chief argument which can be drawn from the present data is a smaller case mortality, but such mortality might be obtained in all probability by specialised medical care as apart from isolation."

The weak point is the assumption that Diphtheria is a uniform disease and that all cases returned as such by a certifying doctor are really suffering from that complaint. We have seen early in the present paper that difference of opinion between two medical men with respect to this disease is found in 26 % of the cases notified. Further, in those prosperous areas where there is an adequate medical staff backed by a well equipped laboratory, bacterial Diphtheria will be included with clinical Diphtheria, that is to say, that a large number of mild cases will appear in the returns and consequently the case mortality will be low. If correction were made for the type of disease prevalent, which could be done by making the case mortality constant, it is possible that the correlation between isolation rate and mortality per 1000 of population would be materially altered. The tacit assumption that sanitation in the popular sense of the word may be a factor in reducing the rate may in some cases be fallacious, as a well equipped sanitary organisation will include an adequate number of officials, suitable laboratory facilities, ample supply of beds for the isolation of doubtful cases and encouragement to local practitioners to notify them. In Districts of this type an increase in the attack rate with the fall in the death rate may have been produced. From these facts it can be concluded that Diphtheria is in much the same position as Scarlet Fever, namely that infection is extremely widely spread and that a large error of omission occurs. It is hardly likely that isolation of a proportion of the cases during a short period of time will materially affect the amount of floating contagion. Direct evidence bearing on the number of contacts infected with the organism and on its distribution amongst a school population will be found in Professor Nuttall's *Text Book*, pages 425-490.

*Age of parent at birth and age of attack.*

The data are as follows:

TABLE XIX.

*Diphtheria. Age of parent at birth of offspring and age of attack—the parent being alive.*

Age of attack	Age of parent at birth													Totals
	18-19	20-21	22-23	24-25	26-27	28-29	30-31	32-33	34-35	36-37	38-39	40-41	42 and over	
0	1	—	—	3	1	1	2	1	5	—	1	1	—	16
1	2	4	6	10	10	7	7	4	5	4	4	3	1	67
2	3	8	5	6	7	10	4	6	1	3	2	2	2	59
3	2	9	3	14	9	15	8	6	8	6	1	3	—	84
4	2	4	8	12	14	7	10	6	8	3	4	2	3	83
5	1	9	12	13	12	13	9	7	6	7	4	5	1	99
6	2	2	4	14	7	10	9	5	3	6	1	1	3	67
7	—	7	9	6	9	5	7	3	6	—	—	—	1	53
8	1	—	3	7	8	1	1	1	2	3	1	1	1	30
9	1	2	2	2	2	3	3	2	—	1	—	—	—	18
10	2	1	4	3	1	1	5	2	2	1	4	—	1	27
11	1	3	4	5	5	4	1	5	1	—	2	2	—	33
12	—	—	2	—	1	—	2	1	—	—	1	1	—	8
13	—	3	—	4	1	3	3	1	2	1	—	2	—	20
14	1	—	4	5	3	1	—	1	—	1	1	—	1	18
15	—	2	1	—	—	1	1	1	1	—	—	—	—	7
16	—	—	—	—	—	—	1	—	—	—	—	—	—	1
Totals	19	54	67	104	90	82	73	52	50	36	26	23	14	690

The coefficients found are:

Age of attack and age of mother at birth  $r = .034 \pm .0272$ .

$\sigma$  Age at attack ... .. = 3.632.

$\sigma$  Age at birth ... .. = 2.913.

$m$  Age of attack for arrays of mother's age = .143.

Corrected ... .. = .0173.

The means are:

Age at birth	Number	Mean
18-21 yrs	63	5.96
22-23 „	64	6.87
24-25 „	104	5.93
26-27 „	90	5.91
28-29 „	82	5.59
30-31 „	73	6.21
32-35 „	102	5.67
36 and over	99	5.86

If we accept the correction for death of parent, the correlation between age of mother at birth and

$$\text{Age of attack of child } r = .05 \pm .03.$$

From this taken by itself no conclusion could be drawn, but in so far as the same trend has been observed in the case of Scarlet Fever the result is suggestive.

The sequences are so irregular that, beyond the slight trend for the age of attack on the average to become later with the late born, little can be said.

#### ENTERIC FEVER.

With respect to Enteric Fever, the data are even more doubtful, as the error of commission is, according to the Metropolitan Asylums Board returns, about 40 %. There is no information as to the error of omission. Practically our heading in this case should read, "The Influence of Age of Parent at Birth of Offspring and Age of Attack in certain disorders of an 'Intestinal Type.'"

As the data were obtained in the same manner as those for Diphtheria and Scarlet Fever, the same defects will occur amongst those attacked late and born of mature parents. Hence on a random basis a negative correlation is expected.

The following figures relative to the epidemic at Lincoln and Basingstoke exemplify the point that multiple cases occur more frequently than chance could account for<sup>1</sup>.

	<i>Lincoln.</i>		<i>Basingstoke (1905).</i>	
	No. of Houses expected to contain	No. of Houses found to contain	No. of Houses expected to contain	No. of Houses found to contain
0 cases	11,233	11,358	1864	1839.5
1 case	905	706	139	151.2
2 cases	36	79	11	6.2
3 „	1	27	1	.2
4 „ and over	1	7	—	—
	12,176	12,177	1997	1997

Goodness in fit,  $P = .045$ .

<sup>1</sup> The example was worked out in class at the Lister Institute. It gives the first five terms of the binomial expansion of  $12176 \left( \frac{1}{12176} + \frac{12175}{12176} \right)^{1006}$ . Naturally the method must be used with reserve owing to variation in size and number of inhabitants of houses.

The features of the distribution are similar to those found in the case of Scarlet Fever, dependent on either milk or spread in the family itself. It must be also admitted that an inherited tendency running in particular families associated with infection from a general source would produce a similar result. The much longer incubation period of Enteric Fever and the sharp rise in the epidemic under consideration, and other reasons mentioned in the official account, leave little doubt that the spread was mainly due to the water supply. To what extent the errors already considered in the case of Scarlet Fever will affect the mean age of attack, can hardly be conjectured. In any case the indefinite nature of the material renders any detailed analysis of little value.

The data are as follows:

TABLE XX.

*Age of mother at birth of offspring and age of attack of  
Enteric Fever. Mother still living.*

Age of attack	Age of parent at birth													Total
	20 and under	21—22	23—24	25—26	27—28	29—30	31—32	33—34	35—36	37—38	39—40	41—42	43—44	
0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1	—	—	—	1	1	—	1	—	—	1	—	—	—	4
2	1	—	1	—	2	—	—	—	—	—	—	—	—	4
3	—	—	1	1	2	2	1	—	—	—	1	1	—	9
4	—	2	1	4	8	1	1	—	1	—	1	1	1	21
5	1	—	3	3	3	1	1	2	—	—	1	—	—	15
6	2	4	3	2	1	4	1	—	1	1	—	1	—	20
7	1	4	2	3	2	3	1	—	—	1	—	—	—	17
8	1	2	2	1	3	2	2	1	—	1	1	1	—	17
9	—	2	—	2	1	—	1	—	—	—	—	1	—	7
10	1	—	1	2	2	1	—	—	—	—	—	1	—	8
11	—	—	2	2	3	2	—	—	1	—	—	—	—	10
12	—	2	2	—	2	1	—	—	1	—	—	—	—	8
13	2	—	1	1	—	2	1	2	2	—	—	3	—	14
14	1	—	1	1	—	—	1	—	1	—	—	—	—	5
15	—	1	—	—	2	—	—	2	1	2	—	—	—	8
16	—	1	—	1	1	1	—	—	—	—	—	—	—	4
17 & over	4	5	6	11	4	5	7	3	1	—	6	1	—	53
Totals	14	23	26	35	37	25	18	10	9	6	10	10	1	224

The constants found were:

$$\sigma_{\text{age of attack}} = 6.1404, \quad \sigma_{\text{age at birth}} = 5.8485.$$

$$r = .0293 \pm .0472.$$



In view of the fact that a negative correlation was expected it seems likely that the earlier born are attacked at a more immature age, or react more characteristically, and are recorded whilst the others are missed.

#### SMALL-POX AND TUBERCULOSIS.

The following diseases, namely Small-pox and Tuberculosis, have only been introduced on account of their intrinsic interest. It should be clearly understood that, owing to the small number of observations available, the results at best can be taken as suggestions only.

##### (1) *Small-pox.*

These data were obtained through the courtesy of Drs Hamer and Millard, under the same conditions as the Scarlet Fever material already described, and hence a negative correlation was to be expected. Small-pox being a more infectious disease than Scarlet Fever, home infection is very probable if the members have not been previously vaccinated. The chances of infection in the later born will be more marked than in the earlier born, and a negative association produced.

The actual data are as follows for father:

TABLE XXI.

*Age of father at birth of offspring and age of attack of Small-pox.  
Father living at time of attack.*

Age of attack	Age of father at birth								Totals
	20 and under	21-24	25-28	29-32	33-36	37-40	41-44	45-48	
0-1	0	5	5	11	5	4	0	1	31
2-3	0	5	11	10	9	6	0	2	43
4-5	0	6	9	10	5	5	2	0	37
6-7	1	5	6	10	7	2	2	0	33
8-9	0	5	8	11	4	7	3	0	38
10-11	1	7	6	9	8	2	0	1	34
12-13	1	4	4	8	3	2	2	0	24
14-15	4	3	11	6	4	3	2	0	33
16-17	0	0	8	7	5	3	2	0	25
18-19	0	2	7	9	6	3	0	0	27
Totals	7	42	75	91	56	37	13	4	325

The constants obtained were:

$$\sigma_{\text{age at birth}} = 1.7. \quad \sigma_{\text{age of attack}} = 2.8.$$

$$\text{Age of father at birth and age of attack } r = -.067 \pm .034.$$

The data for the mother are:

TABLE XXII.

*Age of mother at birth of offspring and age of attack of  
Small-pox.*

Age of attack	Age of mother at birth								Totals
	20 and under	21-24	25-28	29-32	33-36	37-40	41-44	45-48	
0-1	1	5	6	8	4	2	0	1	27
2-3	1	6	12	7	4	3	0	1	34
4-5	1	3	9	7	7	2	1	0	30
6-7	2	7	12	3	2	3	0	0	29
8-9	1	8	5	9	4	4	0	0	31
10-11	3	6	8	8	2	1	0	0	28
12-13	1	3	2	5	2	0	0	0	13
14-15	4	3	9	5	3	1	0	0	25
16-17	0	6	3	5	3	3	1	0	21
18-19	0	2	7	7	1	1	1	0	19
Totals	14	49	73	64	32	20	3	2	257

The constants were:

$$\sigma_{\text{age of attack}} = 2.77, \quad \sigma_{\text{age at birth}} = 1.54,$$

$$r = -0.092 \pm 0.034.$$

$$n_{\text{age of attack for arrays of mother's age}} = 0.0924 \pm 0.035.$$

The regression is effectively linear.

Taking the following coefficients,

Age of father at birth (1) and age of attack (2)

$$r_{12} = -0.0672.$$

Age of father at birth (3) and age of attack (2)

$$r_{23} = -0.0921.$$

Age of father at birth (1) and age of mother at birth (3)

$$r_{13} = -0.7214.$$

Making age of mother constant, the correlation between age of father at birth and age of attack approximates to zero. The same for age of mother at birth and the father being constant,

$${}_1r_{23} = -0.058 \pm 0.032.$$

It would be decidedly unsafe to draw any definite conclusion until more is known relative to the value of the correlation to be expected dependent on home infection, in a population where all families independent of size are grouped together.

(2) *Tuberculosis.*

Turning now to Pulmonary Tuberculosis the data are scanty. It should be noted that in 112 instances given, the Tubercle Bacillus was found in the sputum.

No corrections such as have been described in the previous cases are necessary in these, firstly because information relative to age of parents at birth was obtained irrespective of the fact of the parents being alive or dead, and secondly, there is no evidence as to the relative parts played by general and home infection in the spread of this disease.

In the present state of our knowledge it can be assumed for our purposes that all are equally exposed to risk.

The data are as follows:

TABLE XXIII.

*Age of mother at birth of offspring and age at which Pulmonary Tuberculosis becomes recognisable clinically.*

Age of attack	Age of mother at birth of offspring															Totals
	17-18	19-20	21-22	23-24	25-26	27-28	29-30	31-32	33-34	35-36	37-38	39-40	41-42	43-44	45 and over	
0-8	1	—	—	2	1	1	4	1	—	2	1	1	—	—	—	14
9-12	—	—	1	1	1	2	1	—	—	—	—	—	1	—	—	7
13-16	—	—	1	2	1	2	3	2	—	2	—	—	—	—	—	13
17-20	—	1	1	1	4	2	2	7	—	1	2	1	—	—	—	22
21-24	—	—	1	4	1	3	1	2	2	1	—	2	1	—	1	19
25-28	—	2	1	5	1	3	1	1	3	2	—	1	—	1	—	21
29-32	—	1	1	3	4	1	4	1	4	—	1	1	—	—	—	21
33-36	—	1	1	4	5	4	1	2	1	3	2	2	1	1	1	29
37-40	—	—	1	—	—	2	1	3	2	3	—	1	1	1	—	15
41-44	—	—	1	—	3	—	—	—	1	—	1	—	—	—	—	6
45-48	—	—	—	—	—	1	—	1	—	—	1	—	—	—	—	3
49-52	—	—	—	—	1	—	1	—	—	—	—	—	—	—	—	2
53-56	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1
57-60	—	—	1	—	—	1	—	—	—	—	—	1	—	—	—	3
Totals	1	5	10	22	22	23	19	20	13	14	8	10	4	3	2	176

The constants obtained were:

$$\sigma_{\text{age of attack}} = 2.87. \quad \sigma_{\text{age at birth}} = 3.02.$$

Correlation between age of mother at birth and age of attack

$$r = .09 \pm .05.$$

That is to say, the younger born are probably attacked earlier. If we take Hansen's data as quoted by Dr Greenwood, and fit a straight

line to the number attacked with Tuberculosis and the number expected, in respect to order of birth,

$$r = \cdot 0172 \pm \cdot 0084.$$

$$\text{Mean order expected} = 3\cdot 4236.$$

$$\text{Mean order Tuberculous} = 3\cdot 2817.$$

Hence, on the average, the earlier born are either attacked at younger ages or more frequently than the elder born. The former seems the more likely explanation.

Now bearing in mind that the association between age of mother at birth and age of attack by Tuberculosis is small but positive ( $r = \cdot 09$ ) and for Small-Pox is negative  $r = -\cdot 09$ , it suggests that these two diseases are dissimilar in their incidence. Even should it be supposed that the negative association for Small-pox is dependent on infection in large families, it would still have the same effect on the prevalence of the former disease.

The possibility that the fall in the Phthisis rate from 1840 to the present day may be due to the survival of certain units that would have been killed by Small-pox if prevalent, does seem to be a question worthy of further investigation.

#### *Minor infectious diseases.*

The following table drawn from inquiries of such mothers as attended the examination of children under the Education Act 1898 (Administrative Provisions) leads to conclusions similar to those already drawn from the more serious infectious ailments.

All children were in their 7th year at the time of inquiry and were born in 1903. Each mother was asked whether the child had or had not had the complaints under inquiry.

The data are as follows:

*Incidence of Measles and other diseases on children in the first seven years of life (born in 1903) according to the age of the mother at birth.*

Age of mother at birth of child	Number of inquiries	Number of illnesses per child	Number attacked with measles per 100	Number attacked with whoop- ing cough per 100	Number attacked with chicken-pox per 100
20 and under	21	2.05	97	63	35
21-25	143	2.25	89	59	45
26-30	182	2.1	88	54	41
31-35	92	2.1	82	48	38
36-40	54	1.9	82	40	25
40 and over	41	1.7	80	43	44

NOTE.—The column "illnesses per child" included pneumonia, vague febrile attacks, and other ill-defined conditions.



With the exception of Chicken-pox the percentage incidence falls steadily in all cases as the age of the mother at birth advances. As the age taken was the 7th year, some susceptible children will be attacked at later ages. Hence it is not unfair to assume that for these diseases the later born are attacked at more mature ages or not at all.

#### GENERAL CONCLUSIONS.

Although in many of the instances given the correlations are very small, the concordance does suggest that, with the exception of Small-pox and possibly Chicken-pox, infectious diseases attack the earlier born at younger ages than the later born.

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## THE CHARACTERISTICS OF TUBERCLE BACILLI IN HUMAN BONE AND JOINT TUBERCULOSIS.

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### INTRODUCTION.

THE material on which this enquiry is based consisted of tuberculous tissue, pus, or fluid obtained from the sources specified in Table II, pp. 260-268.

To the surgeons and pathologists attached to the Institutions there named and to the practitioners who have provided us with specimens from private cases we wish to express our cordial thanks for the trouble they have taken in collecting the material, forwarding it to us, and informing us of the essential clinical facts pertaining to it.

During the period of collection, which was from August 1st, 1913, to May 31st, 1914, we have endeavoured to comprise as wide a range of cases as possible without restriction as to age, locality, or other circumstances.

In determining the characteristics of the various strains of tubercle bacilli isolated we have adhered to the methods described in our previous reports on "The Incidence and Bacteriological Characteristics of Tuberculous Infection in Children" and "Localised Tuberculosis in Swine" (*Reports to L. G. B. on Public Health and Medical Subjects*, New Series, Nos. 88 and 91).

### GENERAL RESULTS.

In Table II, pp. 260-268, the cases are set out in the order in which they were received, with the omission of 47 cases yielding negative<sup>1</sup> results; and the type of bacillus obtained from each is stated.

<sup>1</sup> In 23 of these negative cases, egg tubes were inoculated from the spleens, 40 in all, of guinea-pigs killed six weeks after intraperitoneal inoculation with original material. The tubes remained sterile in every instance. This precaution was taken since tubercle bacilli of reduced virulence sometimes fail to produce macroscopic lesions in guinea-pigs, though the bacilli inoculated survive and are recoverable from apparently normal tissues.

In each of the 261 cases which form the basis of the appended statistics, the specimen sent to us consisted of material directly removed from an affected bone or joint, or from an abscess in the neighbourhood of such lesion.

Five additional cases are included in Table II, viz. Nos. 1, 27, 37, 109 and 216. In these, though there was some disease of bone or joint, material directly connected therewith was not available, but specimens from more distal affected sites were sent for examination. We have considered that these five cases are worth putting on record, but have not included them in our statistics as to the types of bacilli found in cases of bone and joint tuberculosis.

The bacilli are described as being either of human or bovine type, or, in a few cases, as atypical, *i.e.* not completely conforming to either of these types. The evidence on which this classification is based, and the characteristics of the "atypical" strains will be found in the subsequent sections of this report.

The results of the investigation are summarised in the following table:

TABLE I.  
*Types of Tubercle Bacilli at Different Age Periods.*

Age period	Number of cases	Human	Bovine	Atypical
0-5 years	47	31	14	2
5-10 "	108	75	31	2
10-16 "	62	52	7	3
16-25 "	15	12	3	—
Over 25 "	29	26	—	3
Total ... ..	261	196	55	10

The percentages of "bovine" cases are:

All ages (55 out of 261)	...	...	21.1 per cent.
Under 10 years (45 out of 155)	...	...	29.0 per cent.
Over 10 years (10 out of 106)	...	...	9.4 per cent.

Only three of the above patients were over 16, the ages of these being 17, 22, and 17 years.

In two cases typical "bovine" strains were obtained from different sites of the body, viz., from a psoas sinus and an empyaema in No. 104, and from a gluteal abscess and an abscess of the zygoma in No. 136.

It will be observed in Table II, in which the cases are presented in the order of receipt, that "bovine" cases crop up irregularly. Percentages computed from a short series might be misleading.



## CHARACTERISTICS OF THE TUBERCLE BACILLI OF HUMAN TYPE.

(1) *Cultures.*

The tubercle bacilli of this group are characterised by the production of luxuriant growth on media containing 5 per cent. of glycerin. On glycerin-agar they produced in from two to three weeks a thick, confluent, wrinkled layer; and the growth on glycerinated potato was abundant and pigmented. Sixteen of the viruses were also grown upon glycerin-broth. They all covered the surface with thick, wrinkled pellicles.

All the strains of tubercle bacilli here included ultimately attained to uniformity in type of growth, though in some cases considerable repetition of the tests was necessary to ensure that a strain had produced the best growth of which it was capable on the test media.

(2) *Virulence for Experimental Animals.*

The original material was always inoculated into guinea-pigs and produced general tuberculosis in almost every case. In the few exceptional cases where the tuberculosis was found to be localised at autopsy, guinea-pigs were tested with culture, the invariable result being the production of generalised tuberculosis.

Each virus was tested on rabbits by the subcutaneous inoculation between the shoulders of 10 mg. (or more) of serum culture. As will be seen from Table III, pp. 272-292, the usual result was that the rabbits, on being killed 100 days after inoculation, were in good health and only slightly affected, showing some deposits of tuberculosis in the lungs but little evidence of dissemination in other organs.

In a relatively small number of cases the rabbits showed disease of a definitely severer type. The lungs were enlarged and extensively replaced by caseous tissue, and in 10 instances the disease proved fatal in less than 100 days. The manifestations of severe infection were confined to the lungs. The lymphatic glands, apart from those directly connected with the site of inoculation, were not affected; neither was the spleen; and there was only slight tuberculosis of the kidneys. In these respects the anatomical features of the disease differed markedly from those produced by inoculation with equal quantities of bacilli of "bovine" type.

TABLE II.  
*Clinical Data and Bacteriological Results.*

No. of case	Initials of patient	Sex	Age in years	Place and institution from which the material was obtained. (In the case of the Alton specimens the patient's home address is added)	Situation of tuberculous lesions	Source of culture isolated		Type of Tubercle bacillus
						Original material	Direct or through G.P.	
1	F. S.	F	12	Queen Mary's Hosp., Carshalton	Hip and glands Wrist (dorsal ganglion) Spine Hip	Cervical gland	Direct	Human
2	W. M.	M	16	St Bartholomew's Hosp., E.C.		Fluid from ganglion	"	"
3	A. H.	F	9	Treloar's Hosp., Alton (London)		Pus from spine	Through G.P.	"
4	A. R.	M	11	Treloar's Hosp., Alton (Oakham, Rutland)		Gluteal abscess	Direct	"
5	A. B.	M	8	Treloar's Hosp., Alton (Chandler's Ford, Hants)	Psoas abscess	Pus from	"	"
6	H. R.	M	6	Treloar's Hosp., Alton (Portsmouth)	Hip Spine	Psoas abscess	Through G.P.	"
7	H. R.	M	4	Treloar's Hosp., Alton (Swanley, Kent)				
8	P. S.	M	9	Treloar's Hosp., Alton (London)	"	"	Direct	"
9	N. R.	F	3	Treloar's Hosp., Alton (Brentford, Middlesex)				
10	E. G.	F	7	Children's Hosp., Gt Ormond St, W.C.	Mastoid Rib Spine	Iliac abscess	Direct	"
11	F. E.	M	5	"		Bone from	Through G.P.	"
12	A. R.	F	4	(Private) Liverpool		Scrapings from	"	"
13	L. H.	F	14	Treloar's Hosp., Alton (Tidworth, Hants)		Psoas abscess	Direct	"
14	L. T.	F	7	Treloar's Hosp., Alton (London)	Psoas abscess Knee Hip Psoas abscess Spine	"	"	"
15	J. B.	M	9	"		Pus from	"	"
16	J. F.	M	20	St Bartholomew's Hosp., E.C.		Synovial membrane	Through G.P.	"
17	L. C.	F	11	Treloar's Hosp., Alton (London)		Pus from	"	"
18	L. D.	M	4	Children's Hosp., Gt Ormond St, W.C.	Psoas abscess Spine	"	Direct	"
19	G. H.	F	6	Treloar's Hosp., Alton (Gosport, Hants)		Psoas abscess	"	Bovine
20	R. M.	M	7	Alexandra Hosp., Queen's Sq., W.C.	Hip Spine Spine Hip Spine	Pus from	"	Human
21	H. S.	M	4	"		"	"	"
22	G. H.	F	6	Queen Mary's Hosp., Carshalton		Psoas abscess	"	"
23	F. W.	M	8	Treloar's Hosp., Alton (London)		Lumbar abscess	Through G.P.	Bovine
24	G. M.	M	4	Children's Hosp., Gt Ormond St, W.C.		Pus from	Direct	Human
25	E. A.	F	5	Treloar's Hosp., Alton (Godstone, Surrey)		Psoas abscess	"	"

26	R. L.	3	Alexandra Hosp., Queen's Sq., W.C.	Epiphysis of radius	Pus from wrist	Direct	Human
27	T. F.	7	" " Ipswich	Hip	Urine	Through G.P.	"
28	T. H.	17	East Suffolk Hosp., Ipswich	Foot	Fifth metacarpal	"	"
29	S. P.	5	Treloar's Hosp., Alton (London)	Spine	Psoas abscess	Direct	Atypical Human
30	E. M.	8	Alexandra Hosp., Queen's Sq., W.C.	Hip	Pus from	"	"
31	C. G.	5	Treloar's Hosp., Alton (London)	"	"	"	"
32	G. H.	10	(Private) Ipswich	Spine	"	"	Atypical Human
33	P. C.	7	Orthopaedic Hosp., Birmingham	Psoas abscess	"	"	"
34	G. R.	50	St Giles' Inf., W.	Lungs, caries of sacrum	Sacral abscess	"	Atypical Bovine
35	H. W.	1½	St Bartholomew's Hosp., E.C.	Mastoid	Tissue from	Through G.P.	"
36	E. L.	5	Alexandra Hosp., Queen's Sq., W.C.	Hip	Pus from	Direct	Human
37	C. S.	35	St Giles' Inf., W.	Lungs, caries of ribs	Abscess in buttock	Through G.P.	"
38	F. C.	30	St Bartholomew's Hosp., E.C.	Psoas abscess	Pus from	Direct	"
39	D. R.	8	Children's Hosp., Gt Ormond St., W.C.	Abscess in thigh	"	"	"
40	L. B.	61	St Bartholomew's Hosp., E.C.	Elbow	Synovial membrane from	Through G.P.	"
41	M. B.	11	Alexandra Hosp., Queen's Sq., W.C.	Hip	Pus from	Direct	"
42	M. D.	40	(Private) Bradford	Sacro-iliac and multiple abscesses	Sacro-iliac and lumbar abscesses	"	Atypical Human
43	W. C.	13	St Bartholomew's Hosp., E.C.	Spine	Psoas abscess	Through G.P.	Human
44	A. U.	8	Children's Hosp., Shadwell, E.	Psoas abscess	Pus from	Direct	"
45	D. R.	9	Alexandra Hosp., Queen's Sq., W.C.	Hip	"	Through G.P.	"
46	R. J.	8	Treloar's Hosp., Alton (London)	Psoas abscess	"	Direct	"
47	R. S.	8	" " "	Hip	"	Through G.P.	"
48	H. B.	35	(Private) Margate	Knee	"	"	"
49	C. Y.	3	St Bartholomew's Hosp., E.C.	Spine	Psoas abscess	Direct	Bovine Human
50	G. L.	8	Treloar's Hosp., Alton (Swansea)	Hip	Pus from	"	"
51	E. S.	11	" " (Salisbury)	"	"	"	"
52	G. D.	12	" " (Cardiff)	Spine	Dressing from lumbar abscess	Through G.P.	"
53	M. B.	9	" " (Portsmouth)	Hip	Dressing from sinus of	"	Human
54	E. P.	17	Sea Bathing Hosp., Margate	Ankle	Scraping from sinus of	"	Bovine
55	R. B.	7	Treloar's Hosp., Alton (London)	Spine	Psoas abscess	"	"
56	V. V.	7	" " "	Hip	Dressing from sinus of	"	"
57	R. R.	9	" " "	Spine	Dressing from lumbar sinus	"	Human
58	W. H.	4	" " "	Hip	Dressing from	"	"
59	A. C.	17	Union Hosp., Sheffield	Knee	Synovial membrane and bone from	"	"
60	R. H.	27	General Inf., Leeds	Ankle	Synovial membrane of	Direct	"

TABLE II—(continued).  
Clinical Data and Bacteriological Results.

No. of case	Initials of patient	Sex	Age in years	Place and Institution from which the material was obtained (In the case of the Alton skin disease the patient's home address is added)	Situation of tuberculous lesions	Source of culture isolated		Type of Tubercle bacillus
						Original material	Direct or through G.P.	
61	M. H.	F	24	(Private) Bath	Sacro-iliac sinuses	Dressing from	Through G.P.	Human
62	L. V.	F	3	Children's Hosp., Gt Ormond St., W.C.	Ankle	Pus from	Direct	"
63	G. J.	M	7	Treloar's Hosp., Alton (Pontypool)	Hip	Gluteal abscess	"	"
64	R. H.	M	4	Fleming Hosp., Newcastle	Spine	Lumbar abscess	"	"
65	E. G.	F	33	(Private) Ipswich	Psoas abscess	Pus from	"	Bovine
66	M. B.	F	10	Alexandra Hosp., Queen's Sq., W.C.	Hip	Bone from	"	Human
67	F. F.	M	36	St Bartholomew's Hosp., E.C.	Psoas abscess	Pus from	"	"
68	A. W.	M	9	Treloar's Hosp., Alton (Penge)	Hip	"	Through G.P.	Atypical
69	J. H.	F	72	General Inf., Leeds	Ankle	Tissue from	Direct	Bovine
70	M. L.	F	22	(Private) Alton	Spine	Dressing from sinus of	Through G.P.	Human
71	W. W.	F	6	Treloar's Hosp., Alton (Romford, Essex)	Hip	Pus from	Direct	"
72	B. L.	F	9	Children's Hosp., Gt Ormond St., W.C.	"	"	"	"
73	E. W.	M	11	Alexandra Hosp., Queen's Sq., W.C.	"	"	"	"
74	S. D.	F	14	Sea Bathing Hospital, Margate	"	"	Through G.P.	Human
75	G. B.	M	8	Treloar's Hosp., Alton (London)	Spine	"	Direct	"
76	F. W.	M	42	(Private) Weston-super-Mare	Tendon sheaths of wrist	Granulations from	Through G.P.	"
77	E. S.	F	2	Queen Mary's Hosp., Carshalton	Multiple bones	Abscess of left int. malleolus	Direct	"
78	P. S.	M	3	Treloar's Hosp., Alton (Birmingham)	Spine	Psoas abscess	"	Bovine
79	T. K.	M	10	Fleming Hosp., Newcastle	Hip	Scraping from	Through G.P.	Human
80	W. M.	M	4	Treloar's Hosp., Alton (Bargood, Monmouth)	"	Pus from	Direct	"
81	C. A.	M	6	Treloar's Hosp., Alton (Lowestoft)	Spine	Psoas abscess	"	Bovine
82	L. B.	F	3	St Bartholomew's Hosp., E.C.	Ankle	Pus from	"	Human
83	J. S.	M	5	Queen Mary's Hosp., Carshalton	Multiple bones and joints	Lumbar abscess	Through G.P.	Atypical
84	A. C.	M	6	Treloar's Hosp., Alton (London)	Spine	Psoas abscess	"	Human
85	W. C.	M	14	Queen Mary's Hosp., Carshalton	Hip	Pus from	Direct	"
86	L. N.	F	4	Children's Hosp., Gt Ormond St., W.C.	"	Gluteal abscess	"	"
87	R. C.	M	7	Royal Inf., Manchester	Psoas abscess	Pus from	"	Bovine
88	R. P.	F	13	Treloar's Hosp., Alton (Aberdovey)	Hip	"	"	Human
89	E. D.	F	8	"	Spine	Psoas abscess	"	Bovine



90	T. S.	5	Treloar's Hosp., Alton (Brighton)	Hip	Pus from	Through G.P.	Human
91	L. P.	9	" " (Cardiff)	Knee	Poptital abscess	Direct	"
92	L. S.	3	" " (Southall, Middlesex)	Spine	Psoas abscess	Through G.P.	Bovine
93	H. B.	8	Treloar's Hosp., Alton (London)	"	Cervical abscess	Direct	Human
94	M. M.	10	" " " Margate	Hip	Pus from	Through G.P.	Bovine
95	H. B.	5	East Cliff Hosp., Margate	Multiple abscesses	Pus near hip	Direct	Human
96	A. G.	11	" " " "	"	Pus from knee	"	"
97	A. F.	17	Sea Bathing Hosp., Margate	Shoulder joint	"	Through G.P.	"
98	G. S.	5	Alexandra Hosp., Queen's Sq., W.C.	Hip	Tissue from	Direct	"
99	C. W.	16	Sea Bathing Hosp., Margate	Knee	Dressing from	Through G.P.	"
100	D. B.	5	Treloar's Hosp., Alton (London)	Psoas sinus	Dressing from psoas abscess	"	"
101	E. C.	3	" " " "	Spine	"	"	"
102	D. S.	8	" " " "	"	Dressing from psoas abscess	"	Bovine
103	J. R.	10	" " (Leicester)	Cervical caries	Dressing from sinus	"	Human
104	L. G.	5	" " (London)	Spine	Dressings from psoas abscess and empyaema	"	Bovine
105	F. I.	10	" " " "	Hip	Dressing from gluteal sinus	"	Human
106	E. W.	10	" " " "	"	Dressing from	"	Bovine
107	V. W.	11	" " (Taunton)	"	"	"	"
108	F. O.	7	" " (Aberbeeg, Monmouth)	Psoas abscess	"	"	"
109	A. W.	8	East Cliff House, Margate	Rib, Multiple abscesses	Abscess of neck	Direct	Human
110	S. D.	71	General Inf., Leeds	Rib	Abscess connected with	"	"
111	W. C.	5	Children's Hosp., Gt Ormond St, W.C.	Knee	Pus from	Through G.P.	Bovine
112	K. S.	7	Southern Hosp., Liverpool	Abscess of instep	"	Direct	Human
113	W. D.	8	Treloar's Hosp., Alton (Sutton, Surrey)	Hip	"	"	"
114	T. F.	41	Royal Inf., Manchester	"	Pus from abscess in thigh	"	"
115	D. L.	3	Treloar's Hosp., Alton (London)	Spine	Psoas abscess	"	"
116	A. J.	4	" " " "	Ankle	Dressing from	Through G.P.	"
117	R. A.	9	" " " "	Spine	"	"	Bovine
118	R. C.	11	" " (Bristol)	Hip	"	"	Human
119	W. G.	13	St Vincent's Home, Pinner, Middlesex	"	Pus from	Direct	"
120	L. A.	6	Children's Hosp., Gt Ormond St, W.C.	Spine	Pus near great trochanter	"	"

TABLE II—(continued).  
*Clinical Data and Bacteriological Results.*

No. of case	Initials of patient	Sex	Age in years	Place and Institution from which the material was obtained. (In the case of the Alton specimens the patient's home address is added)	Situation of tuberculous lesions	Source of culture isolated		Type of Tubercle bacillus
						Abscess of original material	Direct or through G.P.	
121	I. M.	M	4	Royal Hosp., Sheffield	Hip	Pus from	"	"
122	J. W.	M	4	Treloar's Hosp., Alton (Portsmouth)	"	Psoas abscess	"	"
123	L. B.	F	5	Royal Hosp., Sheffield	Multiple bones and joints	Pus from fibula	"	"
124	H. H.	M	7	"	Ulna	Pus from	"	"
125	E. H.	F	6	St Bartholomew's Hosp., E.C.	Psoas abscess	"	"	"
126	J. N.	M	29	Royal Inf., Manchester	Elbow joint	"	"	"
127	T. R.	M	68	Fulham Inf., W.	"	"	"	"
128	W. B.	M	11	Children's Hosp., Gt Ormond St, W.C.	Abscess of thigh	"	"	"
129	S. P.	M	4	"	Knee	"	Through G.P.	"
130	C. C.	M	8	"	Psoas abscess	"	"	"
131	W. W.	M	4	Queen Mary's Hosp., Carshalton	"	"	Direct	"
132	A. N.	F	10	(Private) Manchester	Hip	"	"	Bovine
133	M. C.	M	6	Sea Bathing Hosp., Margate	Hip and pelvis	Lumbar abscess	Through G.P.	Human
134	W. W.	M	15	Alexandra Hosp., Queen's Sq., W.C.	Hip	Pus from	Direct	Bovine
135	E. D.	F	8	Treloar's Hosp., Alton (Kingstone, Somerset)	"	Gluteal abscess and abscess of zygoma	"	"
136	H. B.	M	10	"	"	Scrapings from femur	Through G.P.	Human
137	E. R.	F	9	Fleming Hosp., Newcastle	"	Abscess of	Direct	Atypical
138	L. R.	M	4	"	Knee	Pus from	"	Human
139	R. T.	M	7	Treloar's Hosp., Alton (Wokingham, Berks)	"	Synovial membrane of	Through G.P.	"
140	J. H.	F	5	St Bartholomew's Hosp., E.C.	"	"	Direct	"
141	A. T.	M	27	"	Spine	Psoas abscess	"	"
142	W. N.	M	3	Surgical Home, Baschurch, Shropshire	Hip	Pus from	Through G.P.	"
143	E. W.	F	23	Surgical Home, Baschurch, Shropshire	Shoulder joint	Scraping from	Direct	"
144	A. W.	M	17	Sea Bathing Hosp., Margate	Knee	Pus from	"	"
145	J. O.	M	6	Royal Inf., Manchester	Psoas abscess	"	"	"
146	E. R.	F	52	St Bartholomew's Hosp., E.C.	Knee	Subcrural abscess	"	"
147	G. P.	M	9	Treloar's Hosp., Alton (London)	Rib	Scrapings from	Through G.P.	"
148	A. B.	M	13	Southern Hosp., Liverpool	"	"	"	"

149	S. E.	M	4	Treloar's Hosp., Alton (Worthing)	Spine	Psoas abscess	Direct	Bovine
150	G. S.	M	15	Queen Mary's Hosp., Carshalton	Psoas abscess	Gland from groin	"	Human
151	H. M.	M	56	Cleveland St Inf., W.	Spine	Pus from	Through G.P.	"
152	M. B.	F	6	Treloar's Hosp., Alton (London)	"	Lumbar abscess	Direct	"
153	W. D.	M	10	" Herts)	Hip	Pus from	"	"
154	W. C.	M	38	(Private) Liverpool	Abscess of thigh	"	"	"
155	D. M.	M	14	(Private) Macclesfield	Tendon sheaths of hand	Scrapings from	"	Bovine
156	T. T.	M	11	Surgical Home, Baschureh, Shropshire	Hip	Pus from	"	Human
157	F. L.	M	13	Treloar's Hosp., Alton (London)	Spine and knee	Psoas abscess	"	"
158	P. W.	M	8	"	Spine	"	"	"
159	F. L.	M	9	Royal Inf., Manchester	Knee	Pus from	"	"
160	A. C.	M	3	Treloar's Hosp., Alton (London)	Spine	Psoas abscess	"	Bovine
161	P. C.	F	5	"	Hip	Pus from	"	Human
162	A. M.	M	7	Children's Hosp., GtOrmond St, W.C.	Psoas abscess	"	"	"
163	S. P.	F	8	East Cliff House, Margate	Ankle	"	"	"
164	W. M.	M	25	Royal Inf., Manchester	Knee	"	"	"
165	H. B.	M	12	Sea Bathing Hosp., Margate	"	Abscess in thigh	"	"
166	F. P.	M	27	"	Spine	Psoas abscess	Through G.P.	"
167	J. W.	M	51	Fulham Inf., W.	Elbow	Scrapings from	"	"
168	R. D.	M	9	"	Spine	Psoas abscess	"	"
169	R. D.	M	14	St Bartholomew's Hosp., E.C.	Knee	"	"	"
170	E. B.	M	10	Treloar's Hosp., Alton (Harrow)	"	Tissue from joint	Through G.P.	"
171	A. D.	M	7	Children's Hosp., GtOrmond St, W.C.	Elbow	Bone from	Direct	"
172	J. W.	F	7	Fleming Hosp., Newcastle	Spine	Lumbar abscess	"	"
173	R. H.	F	7	Treloar's Hosp., Alton (London)	Temporal bone	Dressing from	Through G.P.	Bovine
174	B. T.	M	1	(Private) London	Spine	Dorsal abscess	Direct	"
175	R. E.	M	4	"	Knee	Tissue from	Through G.P.	Human
176	H. E.	F	14	Southwark Inf., S.E.	Arm	Abscess of	"	"
177	A. S.	F	7	Metropolitan Hosp., N.E.	Hip	Pus from sinus near	"	"
178	F. P.	M	14	Alexandra Hosp., Queen's Sq., W.C.	"	Pus from	Direct	"
179	A. R.	M	12	"	"	"	"	"
180	W. B.	M	8	Queen Mary's Hosp., Carshalton	Meninges, spine, hip	"	"	"
181	I. P.	M	15	General Inf., Leeds	Knee	Tissue from hip	Through G.P.	"
182	H. M.	M	3	Treloar's Hosp., Alton (Bedford)	Ankle	Dressing from	"	"
183	H. T.	M	3	"	"	"	"	Atypical
184	G. P.	M	10	"	Hip	"	"	Human
185	N. P.	F	7	Glamorgan Treloar's Hosp., Alton (London)	Knee	"	"	"

TABLE II—(continued).  
*Clinical Data and Bacteriological Results.*

No. of case	Initials of patient	Sex	Age in years	Place and institutions from which the material was obtained (In the case of the Alton specimens the patient's home address is added)	Situation of tuberculous lesions	Source of culture isolated		Type of Tubercle Bacillus
						Original material	Direct or through G.P.	
186	J. H.	M	4	Treloar's Hosp., Alton (Maidstone)	Spine	Dressing from lumbar sinus	Through G.P.	Human
187	M. H.	F	13	" " (Edmonton, Middlesex)	Hip	Dressing from	"	"
188	J. B.	M	8	Treloar's Hosp., Alton (London)	Spine	Dressing from dorsal sinus	"	"
189	C. S.	M	10	" " (Dublin)	Hip	Dressing from	"	"
190	W. M.	M	9	" " (London)	"	"	"	"
191	L. G.	F	11	" " "	"	"	"	"
192	A. M.	M	12	" " "	"	"	"	"
193	H. B.	M	13	" " (Ryde, I.O.W.)	"	"	"	Atypical Bovine
194	F. H.	M	5	" " (London)	Ankle	"	"	"
195	D. Q.	F	10	" " (Maidenhead)	Spine	Psoas abscess	"	Human
196	E. W.	F	8	" " (Alton)	"	"	Direct	"
197	N. L.	F	8	" " (South'mpt'n)	Hip	Pus from	"	Bovine
198	J. W.	F	9	" " (Market Harborough)	Spine	Dressing from psoas sinus	Through G.P.	"
199	D. C.	F	9	Children's Hosp., Gt Ormond St, W.C.	Psoas abscess	Pus from	"	Human
200	K. R.	F	10	Fulham Inf., W.	Hip	Scraping from	"	"
201	G. R.	M	5	Children's Hosp., Gt Ormond St, W.C.	"	Pus from	Through G.P.	"
202	L. A.	F	1½	" " "	Ankle	Granulations from	"	"
203	L. B.	F	13	Fleming Hosp., Newcastle	Spine	Pus from	"	"
204	J. R.	M	5	(Private) Manchester	Knee	"	Direct	"
205	R. R.	M	22	Southern Hosp., Liverpool	Wrist	Bone from	"	Bovine
206	F. A.	M	4	Treloar's Hosp., Alton (Birmingham)	Spine	Dorsal abscess	Through G.P.	"
207	E. M.	F	11	" " (Petersfield, Hants)	Knee and hip	Pus from knee	Direct	Human
208	E. H.	F	25	(Private) Liverpool	Knee	Bone from	Through G.P.	"
209	F. B.	M	12	Surgical Home, Baschurch, Shrops.	Spine, elbow	Pus from elbow	Direct	"
210	F. D.	M	7	" " "	Spine	Psoas abscess	"	Bovine
211	J. T.	M	8	Children's Hosp., Gt Ormond St, W.C.	Spine, hip	Pus from hip	"	Human
212	E. R.	M	60	St Bartholomew's Hosp., E.C.	Knee	Tissue from	Through G.P.	"



213	P. M.	4	Metropolitan Hosp., N.E.	Ilium	Pus from	Direct	Human
214	V. H.	6	Treloar's Hosp., Alton (Reading)	Spine and hip	Psoas abscess	"	Bovine
215	I. J.	6	Alexandra Hosp., Queen's Sq., W.C.	Hip	Pus from	"	"
216	G. W.	30	General Hosp., Bristol	Sternum, clavicle, glands	Peritoneal gland	Through G.P.	Human
217	D. T.	4	Treloar's Hosp., Alton (Treharris, Glamorgan)	Tarsus	Dressing from	"	"
218	G. P.	6	Treloar's Hosp., Alton (London)	Knee	Pus from	Direct	"
219	C. W.	36	Surgical Home, Baschurch, Shrops.	Spine	Psoas abscess	"	"
220	E. C.	10	Alexandra Hosp., Queen's Sq., W.C.	Hip	Pus from	"	Bovine
221	K. B.	8	Treloar's Hosp., Alton (London)	Knee	"	"	Human
222	R. M.	7	"	Hip	"	"	Bovine
223	A. H.	4	Sea Bathing Hosp., Margate	Spine	Psoas abscess	"	"
224	E. Y.	11	St Bartholomew's Hosp., E.C. (Private) Liverpool	Hip	Abscess of	"	Human
225	J. C.	6	"	Abscess of hand	Scraping of bone from	Through G.P.	"
226	H. J.	30	Alexandra Hosp., Queen's Sq., W.C.	Knee	Tissue from	"	"
227	F. K.	6	"	Hip	Pus from	Direct	"
228	N. O.	8	"	"	"	"	"
229	H. S.	29	Sea Bathing Hosp., Margate	Spine	Psoas abscess	"	Bovine
230	R. R.	7	Treloar's Hosp., Alton (Edmonton, Middlesex)	"	"	"	Human
231	G. A.		"	"	"	"	"
232	C. W.	9	Treloar's Hosp., Alton (London)	Hip	Pus from	"	"
233	W. G.	13	Fleming Hosp., Newcastle	Spine	Psoas abscess	Through G.P.	"
234	B. B.	11	Treloar's Hosp., Alton (St Albans)	Ankle	Dressing from	"	Bovine
235	D. E.	7	Children's Hosp., Gt Ormond St, W.C.	Hip	Pus from	"	"
236	H. M.	6	Treloar's Hosp., Alton (London)	Spine	Dorsal abscess	Direct	"
237	F. J.	10	St Bartholomew's Hosp., E.C.	Hip	Pus from	"	Human
238	F. B.	9	Treloar's Hosp., Alton (Edmonton, Middlesex)	Spine and ankle	Psoas abscess	"	"
239	C. M.	15	Royal Inf., Manchester	Shoulder	Bone from scapula	Through G.P.	"
240	I. E.	3	Treloar's Hosp., Alton (Tottenham)	Spine	Psoas abscess	Direct	"
241	F. L.	11	" (Aberavon, Glamorgan)	Knee	Pus from	"	"
242	C. Y.	3	Treloar's Hosp., Alton (Goodmayes, Essex)	Spine	Psoas abscess	Through G.P.	Bovine
243	W. R.	19	Queen Mary's Hosp., Carshalton	Knee	Fluid from	"	Human
244	G. R.	22	Royal Inf., Manchester	Hip	Scrapings from	"	"
245	N. F.	9	(Private) Manchester	Spine	Psoas abscess	Direct	Bovine
246	G. M.	9	Treloar's Hosp., Alton (London)	Hip	Pus from	"	Human

TABLE II—(concluded).  
*Clinical Data and Bacteriological Results.*

No. of case	Initials of patient	Sex	Age in years	Place and Institution from which the material was obtained (In the case of the Alton specimens the patient's home address is added)	Situation of tuberculous lesions	Source of culture isolated		Type of Tubercle bacillus
						original material	Direct or through G.P.	
247	J. T.	M	6	Treloar's Hosp., Alton (Bristol)	Psoas abscess	Pus from	Through G.P.	Human
248	F. S.	F	7	" " (London)	Spine	Abscess of	Direct	"
249	A. U.	F	9	" " (Silvertown, Essex)	"	Dressing from lumbar sinus	Through G.P.	"
250	T. H.	M	17	General Inf., Leeds	Ankle	Tissue from	"	Bovine
251	J. T.	M	49	(Private) Liverpool	"	Bone from	"	Human
252	H. C.	M	11	Union Hosp., Sheffield	Spine	Psoas abscess	"	"
253	H. J.	M	68	St Bartholomew's Hosp., E.C.	Sternum	Pus from	Direct	"
254	F. H.	F	21	(Private) Liverpool	Spine	Bone from	Through G.P.	"
255	G. A.	M	5	Treloar's Hosp., Alton (Southampton)	Hip	Pus from	"	Bovine
256	E. D.	M	43	St Bartholomew's Hosp., E.C.	Knee	Fluid from	Direct	Human
257	A. S.	F	11	Fleming Hosp., Newcastle	Metatarsal bone	Piece of	Through G.P.	"
258	N. D.	F	5	" "	Hand	Metacarpal bone	"	Bovine
259	C. W.	F	10	Alexandra Hosp., Queen's Sq., W.C.	Hip	Pus from	Direct	Human
260	E. M.	F	11	Fleming Hosp., Newcastle	Spine	"	"	"
261	E. C.	F	6	King Edward VII Hosp., Cardiff	Hip	"	Through G.P.	"
262	A. S.	F	12	Children's Hosp., Gt Ormond St, W.C.	Psoas abscess	"	"	Atypical
263	T. N.	M	11	Fleming Hosp., Newcastle	Tarsal bone	Piece of	Direct	Human
264	L. G.	F	2	St Bartholomew's Hosp., E.C.	Ear	Tissue from	Through G.P.	"
265	J. M.	M	11	Royal Inf., Manchester	Rib	Tissue from	Direct	"
266	H. B.	M	3	Royal Hosp., Sheffield	Hip	Pus from	"	"

As will be seen from Table III, the cultures which had produced exceptionally severe lung disease in rabbits were recovered, with the object of ascertaining whether this relatively high degree of virulence was maintained or possibly increased. The results show that this was not the case. Cultures made from severely affected lungs and tested on fresh rabbits usually produced a much slighter amount of disease; rarely was the disease as severe as before, and never of greater severity.

It has often been found that, when two or more rabbits were inoculated with the same quantities of the same strain, the disease in the lungs might be severe in one case but slight in the companion animals.

The question arises whether there are intrinsic differences in virulence between different viruses of human type. On the one hand, it may be argued that such differences do exist but, being relatively slight, are only brought into prominence when rabbits of somewhat exceptional susceptibility are used. On the other hand, the exceptionally severe results may be regarded as attributable to rupture of small vessels in the subcutaneous tissues during the operation of inoculation and immediate access to the bloodstream of some portion of the material injected. In support of the latter hypothesis it may be pointed out that the lesions produced correspond with the normal effects of intravenous inoculation of cultures of human type, and that the apparently higher virulence was not maintained after passage in the rabbit.

Whichever of these alternative explanations be adopted, the experimental facts afford no cause for doubting the value of the rabbit as a means of differentiating the human from the bovine type. They serve, however, to emphasise the importance of repeating the experiments when a eugonic culture, inoculated subcutaneously, produces lung disease of exceptional severity.

We here take the opportunity of remarking that our tabulated results of experiments on rabbits, which form the evidence upon which our conclusions are based, may also be found of use in considering the wider practical question, How far can this work of differentiating between "human" and "bovine" tubercle bacilli be incorporated as part of the routine of the general clinical laboratory? Bacteriological work on the tubercle bacillus is always long and tedious, and in routine work it is desirable to make as many short cuts as can be made without sacrifice of accuracy. One common difficulty with the tubercle bacillus is that a "human" strain may not be in full vigour when implanted on the glycerinated test media, and, in consequence, the

resultant growth may be poor. If such a strain happened to produce fairly severe lung disease in a rabbit, there might be a temptation, in hurried work, to make the diagnosis "bovine." It is therefore important to call attention to this danger and to show how it can be guarded against. In view of this object, the lengthy series of experimental data which we have provided will enable other investigators to form their own opinions as to the validity of the rabbit test and the care requisite for its accurate interpretation.

### (3) *Effects of Passage in Rabbits*

In 41 instances cultures were recovered from rabbits which had been used for the virulence tests, and the characters of these passage strains were compared with the characters of the original cultures. With three exceptions, they were found to be identical with the originals. Eighteen of these cultures were also tested on rabbits and, with the same three exceptions, were found to correspond with the human type as regards pathogenicity.

The exceptions were the cultures from the kidneys of Rabbit 527 (virus 37), Rabbit 17 (virus 208) and Rabbit 144 (virus 199). Rabbit 527 was killed 100 days after inoculation and showed a cystic local lesion, moderately severe tuberculosis of the lungs, a tubercle in the spleen, and a few tubercles and projecting nodules in the kidneys. A culture made from one of the kidney nodules was dysgonic in character and fully virulent for two rabbits, which died from typical bovine tuberculosis in 41 and 30 days. The companion to Rabbit 527, inoculated with the same dose, developed only slight disease. Further experiments were instituted. The culture used for inoculation of Rabbit 527 not being available, that from the fellow guinea-pig inoculated with original material was tested on three rabbits, with slight results. A culture from the kidney of one of these rabbits was again passed into two rabbits, again with slight results.

Rabbit 17 (virus 208) was killed 100 days after inoculation and showed only slight tuberculosis. A culture was made from one of two small tubercles in the kidneys. This culture, when tested, was found to be dysgonic and fully virulent for two rabbits which died in 34 and 41 days with typical bovine tuberculosis. Rabbit 18, companion to Rabbit 17, showed similar slight infection; a culture was made from a kidney tubercle and proved to be eugonic, like the original; it was not tested in rabbits. The original guinea-pig culture was re-inoculated



into two rabbits and produced only a small amount of disease; cultures from the lungs and a kidney of one were eugonic.

The culture from Rabbit 144 has been found to be fairly luxuriant but not equal to the human type; in a dose of .01 mg., inoculated intravenously, it has produced in a rabbit generalised tuberculosis resembling the "bovine" disease. The characters of this strain are not yet fully elucidated.

There are three possible explanations of the exceptional results obtained with the kidney cultures of Rabbits 527, 17, and 144, viz. (1) modification in the rabbit, (2) an admixture of bovine with human tubercle bacilli in the original material, (3) accidental infection of the rabbits with bovine tubercle bacilli. The experiments recorded above do not afford basis for a definite decision between these three alternatives.

#### A NOTE ON TABLE III.

In a few cases broth cultures were used for inoculation instead of serum cultures; and the site of inoculation was the subcutaneous tissue over the abdomen instead of between the scapulae. This is the method employed by some of the German investigators, who have rarely obtained severe lesions in rabbits inoculated with bacilli of human type. We tried it for the purpose of comparison with the method we have usually followed. As will be seen from the table, no tangible differences can be made out.

As we were dealing with eugonic cultures we have often exceeded the dose of 10 mg., which is the recognised dose for comparison of "bovine" with "human" virulence. This was done purposely, as it was theoretically possible for the eugonic culture to contain an admixture, perhaps scanty, of dysgonic and highly virulent bacilli; and the larger dose would give the latter element, if present, a better opportunity of manifesting itself. With human tubercle bacilli, subcutaneously inoculated, increase of dose makes little difference to the final result.

Twenty-five rabbits inoculated with one or other of the cultures of human type have been omitted from this table. Of these, 22 died prematurely, from intercurrent disease or other cause, in from 1 to 50 days after inoculation. The tuberculous lesions were only slight, except in one rabbit, inoculated with virus 44, where the lungs were somewhat severely affected.

The remaining three, Rabbits 617, 738 and 93 exhibited post-mortem evidence of accidental infection. The first, which died 24

TABLE III. *Rabbits inoculated with Viruses of "human" type.*

The cultures were all grown on pure serum, except those marked \*, which were grown on broth.

The subcutaneous inoculations were all made between the shoulders, serum cultures being used, except those marked †, which were made over the abdomen, with broth cultures.

No. of case	Source of cultures	Details of Cultures			Mode of inoculation	No. of Rabbit	Weights of Rabbits in grammes		Duration of Experiment (days)	Post-mortem Results						Kidney*
		Age of sub. culture in days	Generation	Total duration in days			Initial	Final		Local lesion	Local glands	Lungs	Bronchial glands	Spleen	Three small tubercles <sup>(a)</sup>	
1	Cervical gland	*25	8th	108	20	402	3,420	2,820	K 101	Caseous	Caseous foci	Discrete tubercles; crepitant	tissue	Nil	Nil	Nil
	"	*25	8th	108	20	403	1,880	1,400	K 101	Caseous	Caseous foci	Partially replaced by caseous tissue	Nil	Nil	Nil	Nil
	"	25	8th	108	10	404	2,840	3,250	K 101	Caseous	Nil	Discrete tubercles and caseation of margins	Nil	Nil	Eight small tubercles	Nil
2	Dorsal ganglion of wrist	*12	6th	90	15	392	2,540	3,000	K 100	Caseous	Nil	A few minute tubercles	Nil	Nil	Nil	Nil
3	"	14	3rd	97	10	401	2,100	2,820	K 100	Caseous	Nil	A few minute tubercles	Nil	Nil	Nil	Nil
	"	11	4th	37	10	391	2,770	3,100	K 100	Cystic	Nil	Partially replaced by caseous tissue	Nil	Nil	One tubercle and a few depressions on surface	Nil
	"	*20	4th	64	20	408	3,570	2,950	K 102	Caseous	Nil	Partially replaced by caseous tissue	Nil	Nil	Nil	Nil
4	"	*20	4th	64	20	409	3,400	2,690	K 102	Caseous	Nil	Some caseous areas	Nil	Nil	Nil	Nil
	"	11	6th	69	10	387	2,860	3,250	K 100	Cystic	Nil	Small tubercles	Nil	Nil	Nil	Nil
	"	*11	6th	69	15	388	1,880	2,800	K 100	Cystic	Nil	Small tubercles	Nil	Nil	Nil	Nil
5	Psoas abscess	7	5th	70	10	393	2,410	2,600	K 100	Cystic	Nil	Partially replaced by caseous tissue	Nil	Nil	Nil	Nil
	"	3	5th	167	10	541	2,180	2,300	K 100	Cystic	Nil	Caseous patches on surface	Nil	Nil	Nil	Nil
	"	3	5th	167	10	542	1,720	1,820	K 100	Caseous	Nil	A few minute tubercles	Nil	Nil	Nil	Nil
6	Pus from hip	10	6th	68	10	389	2,120	2,700	K 100	Caseous	Nil	A few tubercles	Nil	Nil	Nil	Nil
	"	14	5th	160	10	520	2,170	2,550	K 100	Caseous	Nil	A few minute tubercles	Nil	Nil	One grey focus	Nil
7	"	15	4th	52	10	420	3,380	3,250	K 100	Cystic	Nil	Enlarged; extensively caseous	Nil	Nil	A few tubercles	Nil
	"	*15	4th	52	35	421	2,280	1,500	D 86	Caseous ulcer	Caseous	Enlarged; completely solid and caseous	Nil	Nil	A few small tubercles and depressions on surface	Nil

7	Rabbit 421 (lung)	10	2nd	27	11	sub.	619	1,750	1,400	K 100	Cystic	Caseous focus	Small tubercles and margins	Caseous patches on surface and at margins	Caseous	Nil	One grey focus and minute depressions on surface
	"	"	10	2nd	27	11	sub.	620	1,580	1,550	Caseous ulcer	Caseous	Caseous	Caseous	Nil	Nil	A few small tubercles and depressions on surface
8	Psoas abscess	*14	7th	70	20	†sub.	395	2,010	2,300	D 96	Fibrous	Nil	Nil	Nil	Nil	Nil	Nil
	"	*14	7th	70	20	†sub.	396	1,750	2,500	K 100	Caseous	Nil	Nil	Nil	Nil	Nil	Nil
	"	14	7th	70	10	sub.	397	1,600	2,540	K 100	Caseous	Nil	Nil	Extensively replaced by caseous tissue	Caseous	Nil	Nil
9	Psoas abscess	10	3rd	49	10	sub.	418	3,480	1,700	D 90	Cystic ulcer	Caseous foci	Extensively replaced by caseous tissue	Extensively replaced by caseous tissue	Caseous	Nil	A few small tubercles
	"	*10	3rd	49	20	†sub.	419	3,060	2,650	K 100	Caseous	Nil	Partially replaced by caseous tissue	Partially replaced by caseous tissue	Nil	Nil	A few small tubercles
	Rabbit 418 (lung)	10	3rd	35	10	sub.	637	2,620	1,900	K 100	Caseous	Caseous	Partially replaced by caseous tissue	Partially replaced by caseous tissue	Caseous focus	Nil	Several small foci
10	"	10	3rd	35	10	sub.	638	1,150	1,700	K 100	Cystic	Nil	Several small tubercles	Several small tubercles	Nil	Nil	Nil
	Iliac abscess	12	6th	77	10	sub.	405	2,490	3,200	K 101	Cystic	Nil	Small tubercles and margins	Small tubercles and margins	Nil	Nil	Nil
	"	*12	6th	77	20	†sub.	406	2,440	2,270	K 101	Caseous	Nil	Extensively replaced by caseous tissue	Extensively replaced by caseous tissue	Nil	Nil	Nil
	"	*12	6th	77	20	†sub.	407	2,070	2,150	K 101	Caseous	Nil	Extensively replaced by caseous tissue	Extensively replaced by caseous tissue	Nil	Nil	Nil
11	Bone from mastoid	15	4th	46	10	sub.	414	2,600	2,650	K 100	Caseous	Nil	A few minute tubercles	A few minute tubercles	Nil	Nil	Nil
	"	*15	4th	46	36	†sub.	415	1,500	1,900	K 100	Caseous	Caseous foci	A few small tubercles	A few small tubercles	Nil	Nil	Nil
12	Scraping from rib	15	3rd	38	10	sub.	412	2,850	2,670	K 100	Cystic	Nil	Discrete tubercles	Discrete tubercles	Nil	Nil	Nil
	"	*15	3rd	38	60	†sub.	413	1,620	1,970	K 100	Caseous	Caseous foci	Caseous patches	Caseous patches	Nil	Nil	Nil
13	Psoas abscess	10	4th	51	10	sub.	390	2,350	3,020	K 100	Cystic	Caseous focus	Minute tubercles	Minute tubercles	Nil	Nil	Nil
	"	14	4th	143	13	sub.	522	2,570	—	K 100	Cystic	Nil	Discrete tubercles	Discrete tubercles	Nil	Nil	Nil
14	Psoas abscess	13	4th	54	10	sub.	400	1,970	2,420	K 100	Caseous	Nil	One small tubercle	One small tubercle	Nil	Nil	Nil
	"	12	3rd	95	12	sub.	508	3,450	3,270	K 100	Cystic	Nil	A few small tubercles	A few small tubercles	Nil	Nil	Nil
15	Psoas abscess	*13	4th	52	20	†sub.	398	1,980	2,750	K 100	Caseous	Nil	Small tubercles	Small tubercles	Nil	Nil	Nil
	"	13	4th	52	10	sub.	399	1,680	2,750	K 100	Cystic	Nil	Two minute tubercles	Two minute tubercles	Nil	Nil	Nil
16	Synovial membrane of knee	8	4th	37	50	sub.	427	2,420	2,420	K 100	Caseous	Nil	Extensively replaced by caseous tissue	Extensively replaced by caseous tissue	Nil	Nil	A few depressions on surface

TABLE III. *Rabbits inoculated with Viruses of "human" type—(continued).*

No. of case	Source of Cultures	Details of Cultures			Dose (mg.)	Mode of inoculation	No. of Rabbit	Weights of Rabbits in grammes		Duration of Experiment (days)	Post mortem Results			
		Age of sub-culture in days	Generation	Total duration of culture in days				Initial	Final		Local lesion	Local glands	Lungs	Bronchial glands
16	Synovial membrane of knee	9	8th	93	13	sub.	521	2,640	2,500	K 100	Caseous ulcer	Nil	Extensively replaced by caseous tissue	Opaque foci
	Rabbit 427 (lung)	9	5th	38	15	sub.	655	2,080	2,700	K 100	Cystic	Cystic	Minute tubercles	Nil
	" "	9	5th	38	10	sub.	656	2,030	1,640	D 91	Caseous	Nil	Small tubercles	Nil
17	Pus from hip	8	3rd	36	12	sub.	429	2,150	2,400	K 100	Caseous	Nil	A few tubercles	Nil
	" "	15	5th	56	11	sub.	453	2,250	2,850	K 100	Caseous	Nil	A few tubercles	Nil
18	Psoas abscess	9	6th	86	11	sub.	436	2,870	2,450	K 101	Caseous	Nil	A few marginal tubercles	Nil
	" "	9	6th	86	11	sub.	437	2,380	2,400	K 101	Cystic	Nil	One grey nodule	Nil
20	Pus from hip	*11	3rd	47	20	†sub.	410	3,340	3,000	K 102	Cystic	One caseous focus	Extensively replaced by caseous tissue	Nil
	" "	11	3rd	47	10	sub.	411	1,900	1,850	K 102	Caseous ulcer	One caseous nodule	Extensively replaced by caseous tissue	Nil
21	Pus from hip	15	4th	51	10	sub.	416	2,430	2,550	K 100	Cystic	Nil	Scattered tubercles	Nil
	" "	*15	4th	51	45	†sub.	417	2,080	1,070	D 92	Caseous ulcer	One caseous focus	Extensively replaced by caseous tissue	Nil
22	Rabbit 417 (lung)	10	2nd	21	12	sub.	618	1,600	1,550	K 100	Caseous	Nil	Minute tubercles	Nil
	Psoas abscess	10	4th	48	10	sub.	422	3,070	1,600	D 94	Cystic	Caseous nodule	Partially replaced by caseous tissue	Nil
	" "	*24	4th	48	24	†sub.	423	3,150	2,750	K 100	Caseous	Nil	A few small tubercles	Nil
	" "	12	8th	148	12	sub.	580	1,820	2,000	K 100	Cystic	Cystic	Three translucent foci	Nil
24	Pus from hip	8	3rd	65	14	sub.	434	3,230	2,140	D 54	Caseous	Nil	Discrete tubercles	Nil
	" "	8	3rd	65	14	sub.	435	3,760	3,350	K 101	Caseous	Caseous	Discrete caseous tubercles	Nil
25	Psoas abscess	5	8th	101	12	sub.	486	2,210	2,050	K 100	Cystic	Cystic	Caseous patches on surface	Nil
	" "	5	8th	101	15	sub.	487	1,820	1,870	K 100	Cystic	Cystic	A few translucent foci	Nil

Kidneys  
A few nodules, one large and projecting  
Nil  
One small tubercle  
Nil  
Nil  
Nil  
A few tubercles in medulla  
A few tubercles

Spleen  
Nil  
Nil  
Nil  
Nil  
Nil  
Nil  
Nil  
Nil  
Nil

Bronchial glands  
Opaque foci  
Nil  
Nil  
Nil  
Nil  
Nil  
Nil  
Nil  
Nil

Lungs  
Extensively replaced by caseous tissue  
Minute tubercles  
Small tubercles  
A few tubercles  
A few tubercles  
A few marginal tubercles  
One grey nodule  
Extensively replaced by caseous tissue

Local glands  
Nil  
Cystic  
Nil  
Nil  
Nil  
Nil  
Nil  
One caseous focus

Local lesion  
Caseous ulcer  
Cystic  
Caseous  
Caseous  
Cystic  
Caseous  
Cystic  
Caseous

Duration of Experiment (days)  
K 100  
K 100  
D 91  
K 100  
K 100  
K 101  
K 101  
K 102

Weights of Rabbits in grammes  
Initial  
Final  
2,640  
2,500  
2,080  
2,700  
2,030  
1,640  
2,150  
2,400  
2,250  
2,850  
2,870  
2,450  
2,380  
2,400  
3,340  
3,000  
1,900  
1,850  
2,430  
2,550  
2,080  
1,070  
1,600  
1,550  
3,070  
1,600  
3,150  
2,750  
1,820  
2,000  
3,230  
2,140  
3,760  
3,350  
2,210  
2,050  
1,820  
1,870

No. of Rabbit  
521  
655  
656  
429  
453  
436  
437  
410  
411  
416  
417  
618  
422  
423  
580  
434  
435  
486  
487

Mode of inoculation  
sub.  
sub.  
sub.  
sub.  
sub.  
sub.  
sub.  
†sub.  
sub.  
sub.  
sub.  
sub.  
sub.  
sub.  
sub.  
sub.  
sub.  
sub.  
sub.

Dose (mg.)  
13  
15  
10  
12  
11  
11  
11  
20  
10  
10  
45  
12  
10  
24  
12  
14  
14  
12

Generation  
8th  
5th  
5th  
3rd  
5th  
6th  
6th  
3rd  
3rd  
3rd  
4th  
2nd  
4th  
4th  
8th  
3rd  
3rd  
8th

Total duration of culture in days  
93  
38  
38  
36  
56  
86  
86  
47  
47  
51  
51  
21  
48  
48  
148  
65  
65  
101

Age of sub-culture in days  
9  
9  
9  
8  
15  
9  
9  
\*11  
11  
15  
\*15  
10  
10  
\*24  
12  
8  
8  
5

Source of Cultures  
Synovial membrane of knee  
Rabbit 427 (lung)  
"  
Pus from hip  
"  
Psoas abscess  
"  
"  
Rabbit 417 (lung)  
Psoas abscess  
"  
"  
"  
Pus from hip  
"  
Psoas abscess  
"  
"



26	Pus from wrist	5	4th	66	10	sub.	438	2,100	2,070	K 100	Caseous	Nil	Discrete tubercles	Nil	Nil	Nil
	"	5	4th	66	10	sub.	439	1,510	1,690	K 100	Caseous	Caseous	A few translucent tubercles	Nil	Nil	Four small tubercles
27	Urine	8	4th	33	12	sub.	454	2,050	2,120	K 100	Cystic	Nil	A few discrete tubercles	Nil	Nil	Nil
	"	8	4th	33	12	sub.	455	1,770	1,800	K 100	Caseous	Nil	Several tubercles	Nil	Nil	Nil
28	Metacarpal bone	3	4th	57	10	sub.	511	2,050	1,850	K 101	Caseous	Nil	A few translucent tubercles	Nil	Nil	Nil
	"	3	4th	57	10	sub.	512	1,890	1,650	K 101	Caseous	Nil	Several discrete tubercles	Nil	Nil	Nil
30	Pus from hip	9	5th	72	10	sub.	456	2,620	1,500	D 82	Caseous	Nil	Completely replaced by caseous tissue	Nil	Nil	A few minute tubercles and depressions on surface
	"	9	5th	72	30	sub.	457	1,810	1,850	K 100	Cystic	Caseous focus	Caseous patches on surface	Nil	Nil	One minute tubercle
	Rabbit 456 (lung)	10	4th	56	11	sub.	693	1,540	1,670	K 100	Cystic	Nil	A few small caseous foci and slight marginal caseation	Nil	Nil	Nil
	"	10	4th	56	11	sub.	694	1,820	1,600	K 100	Caseous	Caseous	Some superficial caseous areas	Nil	Nil	Eight small tubercles
31	Pus from hip	9	5th	72	10	sub.	462	1,770	2,000	K 100	Cystic	Caseous focus	A few translucent tubercles	Nil	Nil	Nil
	"	9	5th	72	14	sub.	463	2,250	2,000	K 100	Caseous	Caseous focus	A few small tubercles	Nil	Nil	One grey focus
33	Psoas abscess	8	4th	43	14	sub.	424	2,040	2,150	K 100	Caseous	Nil	A few small tubercles	Nil	Nil	One tubercle
	"	8	4th	43	14	sub.	425	1,620	1,250	K 100	Caseous	Nil	A few small tubercles	Nil	Nil	Nil
37	Abscess in buttock	8	4th	50	14	sub.	527 <sup>1</sup>	3,090	2,520	K 100	Cystic	Caseous	Partially replaced by caseous tissue	Nil	One minute tubercle	A few small tubercles and one large and projecting
	"	8	4th	50	14	sub.	528	2,590	2,320	K 100	Cystic	Nil	Some superficial caseous areas	Nil	Nil	A few depressions on surface
	"	11	4th	337	10	sub.	111	1,970	2,050	K 100	Cystic	Nil	Small tubercles on surface	Nil	Nil	Nil
	"	11	4th	337	10	sub.	112	2,340	2,450	K 100	Cystic	Nil	A few translucent foci	Nil	Nil	Nil
	"	11	4th	337	·1	i.v.	115	2,270	2,420	K 100	—	—	A few translucent foci	Nil	Nil	A few small tubercles
	Rabbit 115 (kidney)	7	5th	90	10	sub.	155	1,870	2,000	K 100	Cystic	Nil	A few small tubercles	Nil	Nil	Nil
	"	7	5th	90	10	sub.	156	2,020	2,050	K 100	Caseous	Cystic	A few small tubercles	Nil	Nil	A few scars on surface
38	Psoas abscess	10	5th	52	13	sub.	451	1,890	2,200	K 100	Caseous	Caseous foci	A few small tubercles	Nil	Nil	Nil
	"	10	5th	52	13	sub.	452	1,770	1,600	K 100	Caseous	Caseous	Superficial caseous patches	Nil	Nil	One small tubercle

<sup>1</sup> See p. 270.

TABLE III. *Rabbits inoculated with Viruses of "human" type—(continued).*

No. of case	Source of Cultures	Details of Cultures				Mode of Inoculation	No. of Rabbit	Weights of Rabbits in grammes		Duration of Experiments (days)	Post mortem Results <sup>4</sup>					
		Age of sub-culture in days	Generation	Total duration of culture in days	Dose (mg.)			Initial	Final		Local lesion	Local glands	Lungs	Bronchial glands	Spleen	Kidneys
39	Pus from thigh	9	5th	47	11	sub.	458	1,650	2,450	K 100	Caseous	Cystic	A few translucent tubercles	Nil	Nil	Nil
	"	5	5th	82	12	sub.	545	2,150	2,450	K 100	Cystic	Nil	Several small tubercles	Nil	Nil	A few scars on surface
40	Synovial membrane of elbow	7	6th	86	11	sub.	573	1,900	2,020	K 100	Cystic	Nil	A few translucent areas	Nil	Nil	Nil
	"	7	6th	86	11	sub.	574	1,850	1,920	K 100	Cystic	Nil	Caseous tubercles on surface	Nil	Nil	One small tubercle
41	Pus from hip	7	4th	39	16	sub.	440	2,060	1,850	D 75	Caseous	Caseous nodule	Scattered caseous nodules	Nil	Nil	A few minute tubercles and scars
43	"	7	4th	39	16	sub.	441	1,850	1,890	K 100	Cystic	Nil	Small translucent tubercles	Nil	Nil	Nil
	Psoas abscess	6	4th	44	12	sub.	504	3,630	3,000	K 100	Cystic	Nil	Partially replaced by caseous tissue	Nil	Nil	Two grey foci and a few depressions on surface
44	"	6	4th	44	12	sub.	505	1,880	2,200	K 100	Caseous	Caseous	A few translucent tubercles	Nil	Nil	A few pits on surface
	Psoas abscess	5	6th	89	10	sub.	543	2,050	2,150	K 100	Cystic	Nil	A few translucent tubercles	Nil	Nil	Two small tubercles and superficial scars
	"	5	6th	89	10	sub.	544	1,300	1,250	K 100	Cystic	Caseous	Superficial caseation	Nil	Nil	Several small tubercles
45	Pus from hip	10	3rd	42	11	sub.	488	1,700	1,570	K 100	Caseous	Caseous	Caseous patches on surface and at margins	Nil	Nil	Three minute tubercles
46	"	10	3rd	42	11	sub.	489	1,510	1,770	K 100	Caseous	Nil	A few translucent tubercles	Nil	Nil	Three grey foci
	Psoas abscess	6	4th	68	11	sub.	480	1,870	1,700	K 100	Caseous	Cystic	Scattered translucent tubercles	Nil	Nil	Three minute tubercles
	"	6	4th	68	11	sub.	481	1,930	1,800	K 100	ulcer	Caseous	Small tubercles on surface	Nil	Nil	One minute tubercle
47	Pus from hip	4	3rd	38	11	sub.	484	1,920	1,670	K 101	Cystic	Nil	Numerous shotty tubercles	Nil	Nil	One tubercle
	"	4	3rd	38	15	sub.	485	1,870	1,820	K 101	Cystic	Caseous focus	A few small tubercles	Nil	Nil	Nil

48	Pus from knee	6	5th	74	14	sub.	555	1,740	1,900	K 100	Cystic	Nil	Two translucent foci	Nil	Nil	Nil
50	" " " " from hip	6	5th	74	14	sub.	556	1,640	1,700	K 100	Cystic	Nil	Caseous areas on surface	Nil	Nil	Nil
	" " " " " "	7	3rd	33	10	sub.	446	2,220	2,120	K 100	Cystic	Nil	Almost completely replaced by caseous tissue	Nil	One small tubercle	Nil
51	" " " " " "	7	3rd	33	10	sub.	447	1,450	1,570	K 100	Caseous ulcer	Caseous	Partially replaced by caseous tissue	Nil	Two small tubercles	Nil
	Pus from hip	*13	3rd	53	30	†sub.	468	2,280	2,020	K 100	Cystic	Caseous	Not enlarged but extensively replaced by caseous tissue	Nil	A few grey foci	Nil
52	" " " " " "	13	3rd	53	15	sub.	469	1,680	1,570	K 100	Cystic	Nil	Caseous nodules	Nil	A few grey foci	Nil
	bar abscess	7	3rd	36	12	sub.	531	2,370	2,300	K 100	Caseous ulcer	Caseous	Several translucent tubercles	Nil	A few grey foci	Nil
53	" " " " " "	7	3rd	36	12	sub.	532	1,740	1,700	K 100	Cystic	Nil	Some translucent tubercles	Nil	Two grey foci	Nil
	Dressing from sinus of hip	6	6th	66	11	sub.	565	2,220	2,170	K 101	Cystic	Caseous	A few caseous patches	Caseous	Three grey foci	Nil
	" " " " " "	6	6th	66	11	sub.	566	2,000	1,550	D 83	Caseous	Caseous	Almost completely replaced by caseous material	Caseous	A few caseous foci and one large tubercle	Nil
	Rabbit 566 (bronchial gland)	7	—	40	10	sub.	822	1,820	1,950	K 100	Cystic	Nil	A few translucent foci	Nil	Two tubercles	Nil
57	" " " " " "	7	—	40	10	sub.	823	1,520	1,470	K 100	Cystic	Cystic	A few tubercles	Nil	Nil	Nil
	Dressing from lumbar sinus	6	5th	57	12	sub.	551	2,000	1,900	K 100	Cystic	Cystic	Caseous patches on surface	Nil	Six small tubercles	Nil
58	" " " " " "	6	5th	57	12	sub.	552	1,450	1,620	K 100	Cystic	Nil	A few minute tubercles	Nil	Nil	Nil
	Dressing from hip	11	3rd	68	14	sub.	535	2,170	1,600	D 90	Caseous ulcer	Caseous	Some small tubercles	Nil	One grey focus	Nil
59	" " " " " "	11	3rd	68	14	sub.	536	2,190	2,520	K 100	Caseous	Nil	Superficial caseous patches	Nil	Nil	Nil
	Tissue from knee joint	6	5th	59	12	sub.	550	1,400	2,070	K 100	Caseous	Nil	A few translucent foci	Nil	Nil	Nil
60	" " " " " "	5	5th	73	10	sub.	591	1,770	1,940	K 100	Cystic	Nil	A few translucent foci	Nil	Nil	Nil
	" " " " " "	5	5th	73	10	sub.	592	1,980	1,570	K 100	Caseous	Nil	A few tubercles	Nil	Four grey foci	Nil
	Synovial membrane from ankle	9	5th	46	10	sub.	460	2,570	1,770	K 100	Cystic	Nil	Almost completely replaced by caseous tissue	Grey focus	A few small tubercles	Nil
	" " " " " "	6	8th	116	10	sub.	597	2,000	2,040	K 100	Caseous	Nil	A few translucent foci	Nil	Nil	Nil
	" " " " " "	6	8th	116	10	sub.	598	1,800	1,840	K 100	Cystic	Caseous focus	Partial superficial caseation	Nil	Four small tubercles	Nil
	Rabbit 460 (lung)	7	3rd	48	10	sub.	718	1,550	1,790	K 100	Cystic	Calcareous focus	A few minute tubercles	Nil	Nil	Nil
	" " " " " "	7	3rd	48	10	sub.	719	1,920	1,920	K 100	Cystic	Nil	Caseous nodules	Nil	Two tubercles and a few depressions on surface	Nil

TABLE III. *Rabbits inoculated with Viruses of "human" type—(continued).*

No. of case	Source of Cultures	Details of Cultures				Mode of Inoculation	No. of Rabbit	Weights of Rabbits in grammes		Duration of Experiment (days)	Post-mortem Results					
		Age of sub-culture in days	Generation	Total duration in days	Dose (mg.)			Initial	Final		Local lesion	Local glands	Lungs	Bronchial glands	Spleen	Kidneys
61	Dressing from sacral sinus	5	7th	75	13	sub.	595	1,900	1,890	K 100	Cystic	Caseous focus	Small tubercles	Nil	Nil	Nil
	"	5	7th	75	13	sub.	596	1,700	1,770	K 100	Cystic	Nil	A few small tubercles	Nil	Nil	Grey foci in cortex; pus in pelvis of one
62	Pus from ankle	10	3rd	34	14	sub.	449	2,120	1,920	K 100	Caseous	Caseous	Extensively replaced by caseous tissue	Nil	Nil	Nil
	"	10	3rd	34	14	sub.	450	2,020	2,000	K 100	Caseous	Caseous foci	Superficial caseous patches	Nil	Nil	Nil
63	Gluteal abscess	5	4th	55	11	sub.	478	2,720	2,950	K 100	Caseous	Nil	Extensively replaced by caseous tissue	Oedematous	Nil	Three grey foci
	"	5	5th	230	13	sub.	848	2,170	2,350	K 100	Cystic	Nil	Several small tubercles	Nil	Nil	Two grey foci
	"	5	5th	230	13	sub.	849	2,850	2,020	K 100	Caseous ulcer	Caseous foci	A few superficial and marginal tubercles	Nil	Nil	Two grey foci
65	Psoas abscess	10	4th	59	32	sub.	491	1,820	2,150	K 100	Cystic	Cystic	Small translucent tubercles	Nil	Nil	Nil
	"	4	5th	75	11	sub.	547	2,670	2,590	K 100	Cystic	Nil	Superficial caseous patches	Nil	Nil	Two grey foci
	"	4	5th	75	11	sub.	548	1,900	1,740	K 100	Caseous	Caseous	A few small tubercles	Nil	Nil	Three grey foci
66	Bone from hip	5	4th	50	11	sub.	563	2,120	2,050	K 100	Cystic	Cystic	A few small tubercles	Nil	Two small tubercles	Four tubercles
69	"	5	4th	50	11	sub.	564	1,900	2,050	K 100	Cystic	Nil	Several small tubercles	Nil	Nil	One grey focus
	Tissue from ankle	5	4th	54	10	sub.	559	1,600	1,870	K 100	Caseous	Caseous focus	Nil	Nil	Nil	Nil
70	"	5	4th	54	10	sub.	560	2,200	2,100	K 100	Cystic	Nil	One grey tubercle	Nil	Nil	Nil
	Dressing from sinus of spine	8	7th	81	11	sub.	629	1,930	1,850	K 100	Cystic	Nil	A few small tubercles	Nil	Nil	Nil
	"	8	7th	81	11	sub.	630	2,260	2,220	K 100	Cystic	Nil	A few small tubercles	Nil	Nil	One small tubercle
74	Pus from hip	7	3rd	31	13	sub.	529	1,570	1,700	K 100	Cystic	Caseous focus	One translucent tubercle	Nil	Nil	Nil
	"	7	3rd	31	13	sub.	530	1,840	1,900	K 100	Cystic	Nil	Three translucent tubercles	Nil	Nil	Nil



75	Pus from spine	10	3rd	58	11	sub.	509	2,370	2,140	K 101	Cystic	Nil	Superficial scattered tubercles	Nil	Nil	Nil
	" "	10	3rd	58	12	sub.	510	1,770	1,620	K 101	Caseous	Caseous	Discrete tubercles on surface	Nil	Nil	A few grey foci
76	Tendon sheath of wrist	8	5th	66	11	sub.	587	1,380	1,590	K 100	Caseous ulcer	Nil	A few translucent tubercles	Nil	Nil	Nil
	" "	8	5th	66	11	sub.	588	2,150	—	K 100	Caseous	Caseous focus	Several tubercles on surface	Nil	Nil	A few pits on surface
77	Abscess of malleolus	6	4th	64	13	sub.	539	2,550	2,650	K 100	Caseous	Nil	A few translucent tubercles	Nil	Nil	Nil
	" "	6	4th	64	13	sub.	540	2,400	2,100	D 81	Cystic	Caseous	Small translucent foci	Nil	Nil	One grey focus
79	Scraping from hip	8	6th	82	12	sub.	623	1,620	1,450	K 65	Caseous ulcer	Caseous	Extensively replaced by caseous tissue	Caseous	Nil	A few minute tubercles
	" "	8	6th	82	12	sub.	624	1,670	1,820	K 100	Caseous	Nil	A few small tubercles	Nil	Nil	Nil
	Rabbit 623 (lung)	7	3rd	40	13	sub.	814	1,820	1,620	K 100	Caseous ulcer	Caseous foci	A few translucent tubercles	Nil	Nil	Nil
	" "	7	3rd	40	13	sub.	815	3,020	2,540	K 100	Cystic	Caseous foci	Caseous tubercles on surface and at margins	Nil	Nil	One grey focus
80	Pus from hip	7	3rd	30	10	sub.	466	2,390	2,320	K 100	Cystic	Nil	A few minute tubercles	Nil	Nil	One grey focus
	" "	7	3rd	30	16	sub.	467	1,600	1,470	K 100	Cystic	Nil	Caseous patches on surface	Nil	Nil	Nil
82	Pus from ankle	9	3rd	50	10	sub.	506	3,370	1,850	D 76	Caseous	Nil	Extensively replaced by caseous tissue	Nil	Nil	Several tubercles
	" "	9	3rd	50	10	sub.	507	3,170	2,440	K 100	Caseous	Nil	Completely replaced by caseous tissue	Nil	Nil	A few grey foci
	Rabbit 506 (kidney)	6	3rd	50	10	sub.	711	1,770	1,700	K 100	Cystic	Nil	Several caseous nodules	Caseous	Nil	A few tubercles
	" "	6	3rd	50	10	sub.	712	1,790	1,850	K 100	Cystic	Nil	Several discrete tubercles	Nil	Nil	Three tubercles
84	Psoas abscess	6	6th	56	12	sub.	553	1,350	1,970	K 100	Caseous	Cystic	A few tubercles	Nil	Nil	Nil
	" "	6	6th	56	12	sub.	554	1,390	1,500	K 100	Cystic	Nil	Translucent foci and slightly caseous margins	Nil	Nil	One depressed scar
85	Pus from hip	8	6th	87	11	sub.	581	2,000	2,120	K 100	Cystic	Nil	A few minute tubercles	Nil	Nil	Nil
	" "	8	6th	87	11	sub.	582	2,100	1,800	D 92	Cystic	Nil	Almost entirely replaced by nodular caseous substance	Nil	Nil	A few small tubercles
	Rabbit 582 (lung)	9	6th	76	14	sub.	875	2,106	2,270	K 100	Cystic	Nil	A few small tubercles	Caseous	Nil	Two tubercles
	" "	9	6th	76	14	sub.	876	1,690	1,690	K 100	Cystic	Nil	A few small tubercles	Nil	Nil	Nil
86	Gluteal abscess	8	4th	48	12	sub.	523	2,570	2,300	K 100	Cystic	Caseous	Small tubercles	Nil	Nil	A few grey foci
	" "	8	4th	48	12	sub.	524	1,970	2,020	K 100	Cystic	Cystic	Patches of superficial caseation	Nil	Nil	Nil
88	Pus from hip	5	6th	76	10	sub.	593	1,960	2,090	K 100	Cystic	Nil	Patches of superficial caseation	Nil	Nil	Nil
	" "	5	6th	76	10	sub.	594	1,660	1,950	K 100	Caseous	Cystic	Patches of superficial caseation	Nil	Nil	Four small tubercles

TABLE III. *Rabbits inoculated with Viruses of "human" type—(continued).*

No. of case	Source of Cultures	Details of Cultures			Dose (mg.)	Mode of Inoculation	No. of Rabbit	Weights of Rabbits in grammes		Duration of Experiments (days)	Post mortem Results					
		Age of sub-culture in days	Generation	Total duration of cult. in days				Initial	Final		Local lesion	Local glands	Lungs	Bronchial glands	Spleen	Kidney
90	Pus from hip	12	5th	79	11	sub.	657	1,960	1,850	K 100	Cystic	Nil	Numerous discrete tubercles	Nil	Nil	Three tubercles
91	"	12	5th	79	11	sub.	658	1,660	1,670	K 100	Cystic	Nil	Slight superficial caseation	Nil	Nil	Three grey foci
	Popliteal abscess	8	5th	76	10	sub.	613	1,770	1,970	K 100	Cystic	Nil	A few translucent foci and slight marginal caseation	Nil	Nil	One grey focus
93	"	8	5th	76	10	sub.	614	2,050	2,020	K 100	Cystic	Cystic	Partial superficial caseation	Nil	Nil	A few pits on surface
	Cervical abscess	8	5th	69	11	sub.	585	2,110	2,040	K 100	Caseous ulcer	Caseous focus	Slight superficial caseation	Nil	Nil	Nil
95	"	8	5th	69	11	sub.	586	2,150	2,170	K 100	Cystic	Cystic	A few translucent tubercles	Nil	Nil	Nil
	Pus near hip	12	5th	71	10	sub.	601	2,170	2,070	K 100	Cystic	Nil	Nil	Nil	Nil	Nil
96	"	12	5th	71	10	sub.	602	1,620	1,720	K 100	Cystic	Nil	Nil	Nil	Nil	Nil
	Pus from knee	8	5th	69	13	sub.	583	2,500	2,870	K 100	Cystic	Caseous focus	A few translucent foci and slight marginal caseation	Caseous focus	Nil	One grey focus
97	"	8	5th	69	13	sub.	584	1,720	1,790	K 100	Caseous ulcer	Cystic	A few small caseous nodules	Nil	Nil	Three wedge-shaped foci
	Pus from shoulder joint	3	6th	66	10	sub.	649	1,240	1,500	K 101	Cystic	Caseous	A few translucent foci and slight marginal caseation	Nil	Nil	Three small tubercles
98	"	3	6th	66	10	sub.	650	2,340	2,200	K 101	Caseous	Nil	A few tubercles	Nil	Nil	Nil
	Pus from hip	5	5th	58	11	sub.	557	2,050	1,420	D 68	Caseous	Caseous foci	A few translucent tubercles	Nil	Nil	One grey focus
99	"	5	5th	58	11	sub.	558	1,800	2,190	K 100	Cystic	Cystic	Translucent foci	Nil	Nil	Three minute tubercles
	"	6	8th	150	11	sub.	717	2,000	2,270	K 100	Cystic	Caseous	A few translucent tubercles	Nil	Nil	Nil
Tissue from knee	6	5th	51	10	sub.	635	1,650	1,050	D 86	Cystic	Nil	Superficial caseous patches	Nil	Nil	Nil	Three grey foci
	"	6	5th	51	10	sub.	636	1,670	2,000	K 100	Caseous	Caseous focus	Slight superficial caseation	Nil	Nil	Nil
Rabbit 635 (lung)	10	4th	155	15	sub.	905	2,000	950	D 54	Open sore	Nil	Small grey tubercles	Nil	Nil	Nil	Nil
	"	10	4th	155	15	sub.	906	1,950	1,950	K 100	Caseous ulcer	Caseous foci	A few tubercles and slight marginal caseation	Nil	Nil	One depressed tubercle on surface
"	"	6	5th	203	14	sub.	110	2,500	2,400	K 101	Caseous	Nil	Caseous patches on surface	Nil	Nil	Nil

100	Dressing from psoas sinus	5	5th	70	10	sub.	647	1,620	1,790	K 101	Cystic	Nil	Small tubercles on surface	Nil	Nil	Two small tubercles
101	" " Dressing from psoas abscess	5	5th	70	10	sub.	648	1,600	1,900	K 101	Cystic	Nil	A few small tubercles	Nil	Nil	Nil
103	" " Dressing from cervical sinus	13	7th	74	10	sub.	662	2,200	2,270	K 100	Cystic	Nil	A few grey foci	Nil	Nil	Nil
105	" " Dressing from gluteal sinus	11	6th	65	10	sub.	643	2,220	2,150	K 100	Cystic	Nil	A few small tubercles	Nil	Nil	Nil
109	" " Abscess of neck	13	3rd	40	10	sub.	632	1,550	1,570	K 100	Cystic	Nil	Discrete tubercles on surface	Nil	Nil	Two small tubercles
110	" " Abscess of rib	4	6th	86	10	sub.	633	2,300	2,200	K 100	Cystic	Nil	Superficial caseous patches	Nil	Nil	A few grey foci
112	" " Abscess of instep	12	6th	94	10	sub.	641	1,650	1,640	K 100	Cystic	Nil	Surface partially caseous	Nil	Nil	A few pits on surface
113	" " Pus from hip	6	11th	129	11	sub.	689	1,750	1,920	K 100	Cystic	Nil	A few grey foci	Nil	Nil	One small tubercle
114	" " Pus from abscess in thigh	12	6th	92	10	sub.	639	1,770	1,720	K 100	Cystic	Nil	Discrete nodules on surface	Nil	Nil	Two minute tubercles and a caseous streak
115	" " Psoas abscess	5	6th	98	11	sub.	651	2,450	1,920	K 101	Cystic	Nil	A few minute grey foci	Nil	Nil	Nil
116	" " Dressing from ankle	5	6th	98	11	sub.	652	1,500	1,520	K 101	Cystic	Nil	Partially replaced by caseous tissue	Nil	Nil	Six small tubercles
118	" " Dressing from hip	6	5th	83	10	sub.	687	2,330	2,000	K 100	Cystic	Nil	A few translucent foci	Nil	Nil	Nil
119	" " Pus from hip	6	7th	140	11	sub.	688	1,400	1,420	K 100	Cystic	Nil	A few minute tubercles	Nil	Nil	One tubercle
120	" " Pus near great trochanter	7	6th	63	11	sub.	611	1,800	1,900	K 101	Cystic	Nil	A few minute tubercles	Nil	Nil	Nil
	" " "	7	6th	63	11	sub.	612	1,550	1,270	K 101	Cystic	Nil	Scattered nodules and two caseous patches	Caseous nodule	Nil	A few pits and one small tubercle
													Small tubercles on surface	Nil	Nil	One tubercle
													Nil	Nil	Nil	Nil
													Slight superficial caseation	Nil	Nil	One grey focus
													A few small tubercles	Nil	Nil	Two grey foci

TABLE III. *Rabbits inoculated with Viruses of "human" type—(continued).*

No. of case	Source of Cultures	Details of Cultures				Mode of inoculation	No. of Rabbit	Weights of Rabbits in grammes		Duration of experiments (days)	Post-mortem Results				
		Age of sub-culture in days	Generation	Total duration of culture in days	Dose (mg.)			Initial	Final		Local lesion	Local glands	Lungs	Bronchial glands	Spleen
121	Abscess of hip	6	8th	139	11	sub.	708	1,500	1,720	K 100	Cystic	Nil	Grey nodules on surface	Nil	Nil
	"	6	7th	172	13	sub.	808	1,520	2,050	K 100	Cystic	Cystic	Two small tubercles	Nil	Nil
	"	6	7th	172	13	sub.	809	1,550	1,600	K 100	Caseous ulcer	Nil	Small tubercles on surface	Nil	Nil
122	Pus from hip	7	6th	138	13	sub.	699	2,450	2,270	K 100	Cystic	Caseous	Numerous caseous nodules	Nil	Nil
	"	7	6th	138	13	sub.	700	1,670	1,650	K 100	Cystic	Caseous	A few tubercles	Nil	Nil
	"	9	3rd	55	13	sub.	98	1,950	1,850	K 103	Cystic	Nil	Partial caseation of surface	Nil	Nil
	"	9	3rd	55	13	sub.	99	2,350	2,050	K 103	Caseous	Caseous	A few small tubercles on surface	Nil	Nil
123	Psoas abscess	7	7th	138	10	sub.	720	1,700	1,820	K 100	Cystic	Nil	A few grey foci	Nil	Nil
	"	7	7th	138	10	sub.	721	1,450	1,820	K 100	Caseous	Nil	Small scattered tubercles	Nil	Nil
124	Pus from fibula	7	9th	136	12	sub.	705	1,450	1,670	K 100	Cystic	Nil	A few tubercles	Nil	Nil
	"	7	9th	136	12	sub.	706	1,440	1,650	K 100	Caseous ulcer	Cystic	A few tubercles	Nil	Nil
125	Pus from ulna	7	9th	141	10	sub.	732	2,650	2,320	K 100	Cystic	Nil	A few grey nodules	Nil	Nil
	"	7	9th	141	20	sub.	733	2,300	2,020	K 100	Cystic	Nil	Surface replaced by caseous tissue	Nil	Nil
126	Psoas abscess	7	7th	132	12	sub.	703	1,770	2,190	K 100	Cystic	Nil	A few caseating nodules	Nil	Nil
	"	7	7th	132	12	sub.	704	1,600	1,900	K 100	Cystic ulcer	Cystic	Scattered tubercles	Nil	Nil
127	Pus from elbow joint	7	5th	132	11	sub.	701	2,050	2,370	K 100	Cystic	Caseous	A few tubercles	Caseous focus	Nil
	"	7	5th	132	11	sub.	702	1,620	1,670	K 100	Caseous	Cystic	A few marginal nodules	One tubercle	Nil
128	Pus from hip	7	7th	134	10	sub.	726	1,950	2,120	K 100	Caseous ulcer	Nil	A few minute tubercles	Nil	Nil
	"	7	7th	134	10	sub.	727	1,700	1,900	K 100	Caseous	Nil	A few translucent foci	Nil	Nil
	"														One depressed scar

No. of case

Source of Cultures

Age of sub-culture in days

Generation

Total duration of culture in days

Dose (mg.)

Mode of inoculation

No. of Rabbit

Weights of Rabbits in grammes

Initial

Final

Duration of experiments (days)

Local lesion

Local glands

Lungs

Bronchial glands

Spleen

Kidneys

One caseous focus

Nil

Nil

Nil

A few small tubercles on surface

One tubercle

A few small tubercles

Several small tubercles

Two scars on surface

One tubercle

A few tubercles and pits

Nil

Nil

Nil

Nil

Nil

Nil

Nil

Nil

Nil

Nil

Nil

Nil

Nil



129	Abcess of thigh	7	7th	147	11	sub.	744	1,480	1,470	K 100	Caseous	Caseous focus	A few tubercles on surface	Nil	Nil	Nil
130	Pus " from knee	7	7th	147	11	sub.	745	1,680	1,670	K 100	Caseous	Nil	Small caseous areas	Nil	Nil	Nil
		7	8th	116	13	sub.	734	1,750	1,700	K 100	Cystic	Nil	Grey nodules on surface	Nil	Nil	A few small tubercles and pits; pelvis of one caseous
131	" " Psoas abscess	7	8th	116	13	sub.	735	1,920	1,450	K 100	Cystic	Nil	A few tubercles on surface	Nil	Nil	A few tubercles
		11	8th	130	10	sub.	816	1,600	1,900	D 96	Caseous	Nil	Nil	Nil	Nil	Nil
132	" " Psoas abscess	7	8th	130	10	sub.	817	1,620	1,970	K 100	Caseous	Cystic	Nil	Nil	Nil	Nil
		7	6th	145	15	sub.	739	1,480	1,850	K 100	Caseous	Nil	A few grey foci	Nil	Nil	Nil
		10	8th	228	11	sub.	901	2,370	2,050	K 100	Cystic	Caseous focus	Small tubercles on surface	Nil	Nil	Nil
134	Lumbar abscess	5	6th	129	11	sub.	902	2,250	2,220	K 100	Cystic	Nil	A few small tubercles	Caseous focus	Nil	One grey focus
		7	5th	110	12	sub.	778	2,200	2,100	K 100	Caseous	Nil	A few small tubercles	Nil	Nil	A few scars on surface
137	" " Scrapings from femur	5	6th	129	11	sub.	791	1,500	1,350	K 100	Cystic	Nil	One grey focus	Nil	Nil	Nil
		7	5th	110	12	sub.	779	1,850	950	D 98	Caseous	Caseous focus	A few translucent foci and slight marginal caseation	Nil	Nil	Nil
		8	2nd	21	14	sub.	76	1,840	2,070	K 100	Cystic	Cystic foci	Partially replaced by caseous tissue	Nil	Nil	A few grey foci and pits on surface
		8	2nd	21	14	sub.	77	2,270	2,140	K 100	Cystic	Cystic	A few translucent foci	Nil	Nil	One grey focus
139	Pus from knee	5	6th	150	12	sub.	784	1,990	1,800	K 100	Cystic	Caseous focus	Several small tubercles	Nil	Nil	One small tubercle
		5	6th	150	12	sub.	785	1,440	1,420	D 99	Caseous	Caseous focus	Caseous patches on surface	Nil	Nil	Nil
140	Synovial membrane of knee	7	6th	107	12	sub.	782	1,850	1,700	K 100	Caseous	Nil	A few small tubercles	Nil	Nil	One minute tubercle
		7	6th	107	12	sub.	783	1,770	1,500	K 100	Cystic	Nil	A few caseous areas on surface	Nil	Nil	Nil
141	Synovial membrane of kne	11	7th	119	12	sub.	812	2,110	1,800	K 100	Cystic	Nil	A few small tubercles	Nil	Nil	Two caseous nodules
142	" " Psoas abscess	11	7th	119	12	sub.	813	1,920	2,300	K 100	Cystic	Nil	A few small tubercles	Nil	Nil	Nil
		11	7th	157	13	sub.	820	1,700	1,700	K 100	Caseous	Cystic	Slight marginal caseation	Nil	Nil	Nil
143	" " Pus from hip	11	8th	157	10	sub.	821	1,770	1,700	K 100	Caseous	Cystic	One small tubercle	Nil	Nil	Nil
		11	8th	157	10	sub.	818	1,670	1,800	K 100	Caseous	Cystic	Slightly replaced by caseous tissue	Caseous foci	Nil	Nil

TABLE III. *Rabbits inoculated with Viruses of "human" type—(continued).*

No. of case	Source of Cultures	Details of Cultures				Mode of Inoculation (mg.)	No. of Rabbit	Weights of Rabbits in grammes		Duration of Experiments (days)	Post-mortem Results					
		Age of sub-culture in days	Generation	Total duration of culture in days	Dose			Initial	Final		Local lesion	Local glands	Lungs	Bronchial glands	Spleen	Kidneys
143	Pus from hip	11	8th	157	10	sub.	819	1,790	2,100	K 100	Cystic	Cystic	Translucent foci on surface and slight marginal caseation	Caseous foci	Nil	One grey focus
144	Scrapings from shoulder joint	11	9th	192	12	sub.	859	1,960	2,000	K 100	Caseous	Nil	A few minute tubercles	Nil	Nil	Nil
145	"	11	9th	192	12	sub.	860	1,980	2,190	K 100	Caseous	Nil	One small marginal tubercle	Nil	Nil	Nil
	"	12	7th	162	15	sub.	841	1,500	1,950	K 100	Cystic	Nil	A few translucent foci	Nil	Nil	One grey tubercle
	"	13	10th	191	10	sub.	863	2,070	2,100	K 100	Caseous	Nil	A few small tubercles	Nil	Nil	Two small nodules
	"	13	10th	191	10	sub.	864	2,820	2,220	K 100	Cystic	Nil	Several flat tubercles on surface	Nil	Nil	Nil
146	Psoas abscess	9	9th	195	10	sub.	867	1,750	1,800	K 100	Caseous	Nil	A few translucent foci	Nil	Nil	One tubercle
147	"	9	9th	195	10	sub.	868	1,800	1,900	K 100	Cystic	Nil	Slight marginal caseation	Nil	Nil	One small tubercle
	Subcutaneous abscess	12	7th	159	11	sub.	832	1,720	2,020	K 100	Cystic	Nil	Small caseous patches on surface	Nil	Nil	One minute focus
148	"	12	7th	159	11	sub.	833	1,700	2,020	K 100	Cystic	Nil	Thin caseous layer on surface	Nil	Nil	Three small tubercles
	Scrapings from rib	12	5th	126	10	sub.	836	1,920	2,000	K 100	Cystic	Nil	Discrete tubercles on surface	Nil	Nil	Nil
149	"	12	5th	126	10	sub.	837	2,290	2,420	K 100	Cystic	Nil	Translucent foci and slight marginal caseation	Nil	Nil	Nil
	"	12	7th	154	12	sub.	838	1,890	1,850	K 100	Cystic	Nil	Semi-confluent tubercles on surface	Nil	Nil	One scar on surface
150	Gland from groin	12	7th	154	12	sub.	839	2,970	2,450	K 100	Cystic	Nil	Caseous patches on surface	Nil	Nil	One grey tubercle
151	"	12	7th	154	12	sub.	839	2,970	2,450	K 100	Cystic	Nil	A few grey tubercles	Nil	Nil	Nil
	Pus from spine	8	—	115	12	sub.	844	2,170	2,400	K 100	Cystic	Nil	Grey foci on surface and slight marginal caseation	Nil	Nil	A few grey foci
152	"	8	—	115	12	sub.	845	2,200	2,000	K 100	Cystic	Nil	Grey foci on surface and slight marginal caseation	Nil	Nil	Nil
	Lumbar abscess	5	7th	152	11	sub.	842	1,870	2,100	K 100	Cystic	Nil	A few small tubercles	Nil	Nil	Nil
153	"	5	7th	152	11	sub.	843	1,620	1,070	D 65	Caseous ulcer	Nil	A few small nodules	Nil	Nil	Nil
	"	5	7th	152	11	sub.	843	1,620	1,070	D 65	Caseous ulcer	Nil	A few small nodules	Nil	Nil	Nil
154	Pus from hip	11	10th	179	13	sub.	855	2,970	2,850	K 100	Caseous	Caseous	A few grey foci	Nil	Nil	Nil
	"	11	10th	179	13	sub.	856	2,320	2,150	K 100	Caseous	Caseous	A few grey foci	Nil	Nil	A few small tubercles
155	"	11	10th	179	13	sub.	856	2,320	2,150	K 100	Caseous	Caseous	A few grey foci	Nil	Nil	A few small tubercles
	Abscess of thigh	7	7th	150	10	sub.	830	2,020	1,220	K 100	Caseous	Cystic	Minute tubercles	Nil	Nil	Nil
156	"	7	7th	150	10	sub.	831	1,770	1,900	K 100	Caseous	Cystic	A few minute tubercles	Nil	Nil	Nil

156	Pus from hip	9	6th	181	12	sub.	871	1,670	1,870	K 100	Caseous	Nil	Small caseous areas on surface	Nil	Nil	Nil
	"	9	6th	181	12	sub.	872	1,990	1,530	K 100	Caseous ulcer	Nil	Thin caseous patches on surface	Nil	One small tubercle	One small tubercle
157	Psoas abscess	9	9th	181	12	sub.	873	1,940	2,020	K 100	Cystic	Cystic	Slight marginal caseation and a few grey tubercles on surface	Nil	One small tubercle	One small tubercle
	"	9	9th	181	12	sub.	874	2,200	2,220	K 100	Cystic	Nil	Marginal caseation and grey patches on surface	Nil	One grey focus	One grey focus
158	Psoas abscess	8	7th	148	12	sub.	846	2,650	1,750	K 100	Caseous	Nil	A few translucent foci	Nil	Nil	Nil
159	" from knee	8	7th	148	12	sub.	847	1,470	1,570	K 100	Caseous	Nil	Nil	Nil	Nil	Nil
	"	7	6th	147	13	sub.	834	1,890	1,950	K 100	Cystic	Nil	One translucent tubercle	Nil	One grey focus	One grey focus
	"	7	6th	147	13	sub.	835	2,170	2,550	K 100	Cystic	Nil	A few translucent foci	Nil	Nil	Nil
161	Pus from hip	13	7th	175	12	sub.	865	1,500	1,920	K 100	Cystic	Nil	A few flat tubercles on surface	Nil	Nil	Nil
	"	13	7th	175	12	sub.	866	1,500	1,670	K 100	Caseous	Nil	A few small tubercles	Nil	Nil	Nil
162	Psoas abscess	9	8th	178	12	sub.	869	1,770	1,970	K 100	Caseous	Nil	A few discrete tubercles	Nil	Nil	Nil
	"	9	8th	178	12	sub.	870	2,070	1,900	K 100	Cystic	Nil	A few small caseous patches	Caseous	Four small tubercles	Four small tubercles
163	Pus from ankle	11	7th	168	10	sub.	857	2,150	2,050	K 100	Caseous	Cystic	A few small yellow tubercles	Nil	Nil	Nil
	"	11	7th	168	10	sub.	858	1,980	2,200	K 100	Cystic	Caseous	A few greyish tubercles	Nil	One grey focus	One grey focus
164	Pus from knee	13	8th	167	12	sub.	861	1,850	2,120	K 100	Cystic	Nil	Several small tubercles	Nil	Nil	Nil
	"	13	8th	167	12	sub.	862	1,650	920	D 62	Cystic	Caseous	A few yellow tubercles	Nil	Nil	Nil
165	Abscess in thigh	5	8th	205	16	sub.	919	1,950	2,020	K 103	Cystic	Caseous	Caseous patches on surface and at margins	Nil	Two depressed tubercles	Two depressed tubercles
	"	5	8th	205	16	sub.	920	1,750	2,020	K 103	Caseous	Caseous	A few small tubercles	Nil	Two small tubercles	Two small tubercles
166	Psoas abscess	6	8th	176	15	sub.	923	2,170	2,450	K 103	Caseous	Nil	A few tubercles and slight marginal caseation	Nil	Nil	Nil
	"	6	8th	176	15	sub.	924	1,950	2,000	K 103	Caseous	Nil	A few tubercles and slight marginal caseation	Nil	Nil	Nil
167	Scrapings from elbow	6	6th	170	13	sub.	925	1,870	2,000	K 103	Cystic	Caseous	Small tubercles on surface	Nil	Two tubercles	Two tubercles
	"	6	6th	170	13	sub.	926	2,320	2,020	K 103	Caseous	Caseous	Small caseous areas on surface	Caseous	Minute pits on surface	Minute pits on surface
168	Psoas abscess	6	10th	176	15	sub.	951	2,280	1,650	K 100	Caseous ulcer	Caseous	One translucent focus	Caseous	Nil	Nil
	"	6	10th	176	15	sub.	952	1,910	2,150	K 100	Cystic	Nil	One small tubercle	Nil	Nil	Nil
169	Pus from knee	6	9th	204	12	sub.	941	1,770	2,000	K 103	Caseous	Caseous	A few translucent tubercles on surface	Caseous	Nil	Nil

TABLE III. *Rabbits inoculated with Viruses of "human" type—(continued).*

Details of Cultures													Post mortem Results				
No. of case	Source of Cultures	Age of sub-culture in days	Generation	Total duration of cultivation in days	Dose (mg.)	Mode of inoculation	No. of Rabbit	Weights of Rabbits in grammes		Duration of Experiments (days)	Post mortem Results						
								Initial	Final		Local lesion	Local glands	Lungs	Bronchial glands	Spleen	Kidneys	
169	Pus from knee	6	9th	204	12	sub.	942	1,950	1,420	D 102	Cystic	Caseous	Almost completely replaced by caseous tissue	Nil	Nil	A few minute tubercles	
	Rabbit 942 (lung)	8	4th	73	12	sub.	137	2,280	1,850	K 101	Cystic	Caseous	Numerous discrete caseous tubercles	Nil	Nil	A few small tubercles	
	"	8	4th	73	12	sub.	138	1,970	1,300	D 57	Caseous	Nil	Small caseous tubercles on surface	Nil	Nil	Nil	
170	Pus from knee	4	9th	204	12	sub.	939	1,750	1,900	K 103	Cystic	Cystic	Some small tubercles on surface	Nil	Nil	Nil	
	"	4	9th	204	12	sub.	940	1,470	1,470	K 103	Caseous	Caseous focus	A few translucent foci on surface	Nil	Nil	Nil	
171	Tissue from hip	11	6th	185	12	sub.	11	1,550	2,000	K 101	Caseous	Nil	A few translucent foci	Nil	Nil	Two tubercles	
	"	11	6th	185	12	sub.	12	2,020	2,150	K 101	Cystic	Nil	Scattered grey tubercles on surface	Nil	Nil	Pus in pelvis of one	
172	Bone from elbow	6	8th	202	15	sub.	921	2,470	2,400	K 103	Caseous	Nil	A few caseous tubercles	Nil	One minute tubercle	Nil	
	"	6	8th	202	15	sub.	922	1,850	2,050	K 103	Cystic	Nil	Minute tubercles on surface and slight marginal caseation	Nil	Nil	Nil	
173	Lumbar abscess	4	11th	203	12	sub.	937	1,740	1,820	K 103	Cystic	Nil	A few minute tubercles	Nil	Nil	Nil	
176	"	4	11th	203	12	sub.	938	1,770	1,670	K 103	Caseous	Nil	A few small tubercles	Nil	Nil	Nil	
	Tissue from knee	6	4th	176	13	sub.	945	2,220	1,420	K 100	Caseous ulcer	Caseous focus	Several tubercles on surface	Nil	Nil	Nil	
177	"	6	4th	176	13	sub.	946	2,450	2,570	K 100	Cystic	Nil	Small tubercles on surface	Nil	Nil	Nil	
	Abscess in arm	6	7th	176	16	sub.	949	1,850	1,800	K 100	Caseous	Nil	Almost completely replaced by caseous tissue	Nil	Nil	Nil	
	"	6	7th	176	16	sub.	950	1,900	2,020	K 100	Cystic	Nil	A few small tubercles on surface	Nil	Nil	Nil	
178	Pus from hip sinus	8	7th	184	13	sub.	995	1,940	2,100	K 102	Caseous	Nil	A few small tubercles	Nil	Nil	Nil	
	"	8	7th	184	13	sub.	996	2,650	2,420	K 102	Cystic	Nil	Two small tubercles	Nil	Nil	One grey focus	
179	Pus from hip	10	9th	211	15	sub.	985	1,870	2,170	K 100	Caseous	Nil	Slight marginal caseation	Nil	Nil	Nil	
	"	10	9th	211	15	sub.	986	1,570	1,770	K 100	Cystic	Nil	A few translucent foci and slight marginal caseation	Nil	Nil	Nil	



180	Pus from hip	5	7th	97	10	sub.	728	1,800	1,900	K 100	Cystic	Caceous	A few small tubercles	Nil	Nil	Nil
181	" " " hip	5	7th	97	10	sub.	729	1,570	1,550	K 100	Cystic	Nil	One nodule and two tubercles	Nil	Nil	Nil
	Tissue from hip	7	8th	161	12	sub.	965	1,400	1,020	K 100	Caceous	Caceous	Partial caseation of surface	Caceous	Nil	Pus in pelvis of one
	" "	7	8th	161	12	sub.	966	1,620	1,370	D 66	Caceous	Caceous	A few small tubercles	Nil	Nil	A few small tubercles
	" "	7	9th	235	14	sub.	121	2,500	2,400	K 100	Cystic	Nil	Extensive superficial caseation	Nil	Nil	A few small tubercles
	" "	7	9th	235	14	sub.	122	2,020	2,150	K 100	Cystic	Cystic	Small discrete tubercles on surface	Nil	Nil	A few small tubercles
182	Dressing from knee	6	8th	159	14	sub.	947	2,200	2,120	K 100	Cystic	Nil	Several tubercles on surface	Nil	Nil	One small tubercle
184	" " "	6	8th	159	14	sub.	948	1,900	2,170	K 100	Cystic	Nil	Small tubercles on surface	Nil	Nil	One tubercle
184	Dressing from hip	6	5th	161	15	sub.	953	2,250	2,050	K 100	Caceous	Nil	Extensive superficial caseation	Caceous	Nil	Three small tubercles
	" "	6	5th	161	15	sub.	954	1,120	1,800	K 100	Cystic	Nil	Caceous patches and discrete tubercles on surface	Nil	Nil	Nil
185	Dressing from knee	8	10th	176	12	sub.	997	1,570	1,870	K 102	Caceous	Nil	Surface partially replaced by network of caseous tissue	Nil	Nil	One small tubercle
	" "	8	10th	176	12	sub.	998	1,690	1,800	K 102	Caceous	Caceous	A few small tubercles	Nil	Nil	Two small tubercles
186	Dressing from lumbar sinus	5	3rd	161	12	sub.	958	2,100	2,290	K 100	Cystic	Nil	Grey foci on surface and slight marginal caseation	Nil	Nil	Nil
	" "	10	5th	189	14	sub.	78	2,020	1,920	K 100	Cystic	Nil	Discrete tubercles and patches on surface	Nil	Nil	Nil
187	Dressing from hip	5	8th	172	16	sub.	9	1,550	1,720	K 101	Caceous	Nil	A few small tubercles	Nil	Nil	Nil
188	" " "	5	8th	172	16	sub.	10	1,870	2,070	K 101	Caceous	Nil	A few small tubercles	Nil	Nil	Nil
188	Dressing from dorsal sinus	5	7th	160	14	sub.	961	1,820	2,050	K 100	Caceous	Cystic	Two minute foci	Nil	Nil	Nil
	" "	5	7th	160	14	sub.	962	1,800	1,920	K 100	Cystic	Nil	Extensive superficial caseation	Nil	Nil	A few small tubercles
189	Dressing from hip	10	5th	80	10	sub.	810	1,780	2,300	K 100	Cystic	Nil	A few translucent foci and slight caseation of margins	Nil	Nil	Nil
190	" " "	10	5th	80	10	sub.	811	1,760	1,700	K 100	Cystic	Nil	A few small tubercles	Nil	Nil	Nil
	Dressing from hip	5	7th	172	11	sub.	959	1,880	1,300	D 87	Cystic	Opaque foci	A few tubercles	Nil	Nil	Nil
	" "	5	7th	172	11	sub.	960	1,750	1,620	K 100	Caceous	Nil	Grey foci on surface and slight marginal caseation	Nil	Nil	One small tubercle
191	Dressing from hip	5	6th	167	11	sub.	13	2,040	2,350	K 101	Cystic	Nil	A few small tubercles	Nil	Nil	Nil
	" "	5	6th	167	11	sub.	14	1,550	1,650	K 101	Caceous	Caceous	A few small tubercles	Nil	Nil	Nil

TABLE III. *Rabbits inoculated with Viruses of "human" type—(continued).*

No. of case	Source of Cultures	Details of Cultures				Mode of Inoculation	No. of Rabbit	Weights of Rabbits in grammes		Duration of Experiments (days)	Post-mortem Results			
		Age of sub-culture in days	Generation	Total duration of cultivation in days	Dose (mg.)			Initial	Final		Local lesion	Local glands	Lungs	Bronchial glands
195	Psoas abscess	11	5th	170	15	sub.	19	2,190	2,170	K 100	Caseous	Caseous foci	A few caseous tubercles	Nil
196	" "	11	5th	170	15	sub.	20	1,890	2,120	K 100	Caseous	Nil	One small tubercle	Nil
	Psoas abscess	11	8th	212	15	sub.	23	1,900	2,020	K 100	Caseous	Nil	A few small tubercles	Nil
	" "	11	8th	212	15	sub.	24	1,800	1,920	K 100	Caseous	Caseous foci	Numerous small tubercles on surface	Nil
199	Psoas abscess	10	9th	179	13	sub.	62	2,200	2,190	K 101	Caseous	Cystic	Extensively replaced by caseous tissue	Nil
"	" "	10	9th	179	13	sub.	63	1,850	2,000	K 101	Caseous	Caseous focus	Small tubercles	Nil
	Rabbit 62 (kidney)	8	3rd	57	20	sub.	143	2,300	2,050	D 62	Cystic	Nil	A few small tubercles on surface	Nil
200	Rabbit 63 (kidney)	8	3rd	57	15	sub.	144	2,400	2,370	K 100	Caseous	Nil	Soft caseous nodules	Nil
	Scraping from hip	11	7th	179	14	sub.	982	1,700	1,850	K 101	Cystic	Nil	Discrete tubercles on surface	Nil
	" "	16	9th	214	14	sub.	102	2,550	2,800	K 103	Cystic	Nil	A few small grey tubercles on surface	Nil
201	Pus from hip	5	9th	177	13	sub.	955	2,020	1,200	D 76	Caseous	Caseous	Partially replaced by caseous tissue	Nil
"	" "	5	9th	177	13	sub.	956	2,100	2,250	K 100	Cystic	Caseous	Nil	Two minute tubercles
	" "	4	12th	272	16	sub.	125	2,250	2,420	K 101	Cystic	Nil	Slight marginal caseation	Nil
	" "	4	12th	272	16	sub.	126	1,720	1,450	K 101	Cystic	Nil	Several grey tubercles on surface	Nil
202	Tissue from ankle	11	6th	161	15	sub.	973	1,690	1,900	K 100	Caseous	Cystic	Nil	Caseous foci
	" "	11	6th	161	15	sub.	974	1,390	1,690	K 100	Cystic	Caseous	Very slight marginal caseation	Nil
203	Pus from spine	11	7th	175	11	sub.	977	2,140	2,300	K 101	Cystic	Caseous foci	Extensively replaced by caseous tissue on surface	Nil
	" "	11	7th	175	11	sub.	978	1,800	1,920	K 101	Caseous	Nil	Small tubercles on surface	Nil
"	" "	11	7th	175	11	sub.	978	1,800	1,920	K 101	Caseous	Nil	Small tubercles on surface	Nil
	" "	11	7th	175	11	sub.	978	1,800	1,920	K 101	Caseous	Nil	Small tubercles on surface	Nil
	" "	11	7th	175	11	sub.	978	1,800	1,920	K 101	Caseous	Nil	Small tubercles on surface	Nil

Spleen  
Nil  
Two minute foci  
Nil  
Nil  
Nil  
Nil  
A few small tubercles  
One small tubercle  
Nil  
Nil  
A few tubercles  
Nil  
A few scars on surface  
Two minute tubercles  
Nil  
A few small tubercles  
Small scars on surface  
Nil  
Nil  
Nil  
Several small tubercles, some projecting on surface  
Nil

204	Pus from knee	5	11th	202	13	sub.	15	1,890	2,070	K 101	Cystic	Cascous foci	Numerous small tubercles on surface	Nil	Nil	One tubercle
207	"	5	11th	202	13	sub.	16	1,260	1,720	K 101	Cystic	Cascous	A few small tubercles	Nil	Nil	Nil
	Pus from knee	11	8th	196	14	sub.	971	2,370	1,840	K 100	Cystic	Nil	Grey tubercles on surface	Nil	Nil	Nil
	"	11	8th	196	14	sub.	972	1,740	1,940	K 100	Cystic	Nil	Superficial tubercles	Nil	Nil	Two small tubercles
208	Bone from knee	11	8th	175	11	sub.	17	2,300	2,350	K 100	Cystic	Nil	Discrete nodules on surface	Nil	Nil	Two small tubercles
209	"	11	8th	175	11	sub.	18	2,170	2,140	K 100	Cystic	Cascous focus	Small grey tubercles on surface	Nil	Nil	Two small tubercles
	"	5	8th	266	17	sub.	185	1,940	1,950	K 100	Cascous	Nil	Cascous patches on surface and a few nodules in substance	Nil	Nil	Scars on surface and small tubercles
	"	5	8th	266	17	sub.	186	1,960	1,950	K 100	Cascous ulcer	Cascous	A few small tubercles on surface	One cascous	Nil	Nil
210	Pus from elbow	11	8th	193	14	sub.	983	1,990	1,950	K 101	Cascous	Nil	Cascous tubercles on surface	Nil	Two tubercles	One tubercle
	"	11	8th	193	14	sub.	984	1,790	1,870	K 101	Cascous	Cascous	A few grey foci and one cascous nodule	Cascous	Nil	Nil
	"	11	8th	189	14	sub.	975	1,820	2,000	K 100	Cystic	Nil	A few tubercles on surface	Cascous	Nil	One tubercle
212	"	11	8th	189	14	sub.	976	1,490	1,320	K 100	Cascous	Cascous	A few translucent foci	Nil	Nil	Nil
	Tissue from knee	11	6th	155	15	sub.	967	1,700	1,970	K 100	Cascous	Cascous	One translucent tubercle	Nil	Nil	Nil
	"	11	6th	155	15	sub.	968	2,370	2,450	K 100	Cystic	Cystic	Small tubercles on surface	Cascous	Nil	A few tubercles
213	Pus from ilium	11	8th	188	14	sub.	979	2,550	2,270	K 101	Cystic	Cystic	Thin cascous layer on about half of surface	Nil	Nil	Two tubercles
216	"	11	8th	188	14	sub.	980	2,090	2,220	K 101	Cascous	Nil	A few small cascous foci	Nil	Nil	Nil
	Peritoneal gland	7	7th	156	15	sub.	7	1,990	2,020	K 101	Cascous	Nil	A few translucent foci	Nil	Nil	One grey focus
	"	7	7th	156	15	sub.	8	1,740	1,670	K 101	Cascous	Nil	A few small tubercles	Nil	Nil	Nil
217	Dressing from tarsus	7	6th	146	14	sub.	993	2,350	2,340	K 102	Cascous	Nil	A few small tubercles on surface	Nil	Nil	Two depressed tubercles
	"	6	7th	223	14	sub.	109	2,020	2,350	K 101	Cystic	Cystic	Some small tubercles	Nil	One minute tubercle	A few grey foci
	"	7	7th	183	11	sub.	999	1,690	1,650	K 102	Cascous	Nil	Three grey foci	Nil	Nil	Nil
218	Pus from knee	7	7th	183	11	sub.	1	1,970	2,250	K 102	Cascous	Nil	A few tubercles	Nil	Nil	One tubercle
	"	11	6th	181	14	sub.	970	1,700	1,820	K 100	Cascous	Nil	Surface of lungs partially replaced by cascous tissue	Nil	Nil	One small tubercle
	Psoas abscess	6	7th	230	15	sub.	116	2,170	2,420	K 100	Cascous ulcer	Cascous	Discrete tubercles on surface	Nil	Nil	Nil
221	Pus from knee	8	6th	177	14	sub.	987	1,940	2,000	K 100	Cystic	Nil	Extensively replaced by cascous tissue	Nil	Nil	Nil

TABLE III. *Rabbits inoculated with Viruses of "human" type—(continued).*

No. of case	Source of cultures	Details of Cultures				Mode of inoculation	No. of Rabbit	Weights of Rabbits in grammes		Duration of Experiment (days)	Post-mortem Results			
		Age of sub-culture in days	Generation	Total duration of culture in days	Dose (mg.)			Initial	Final		Local lesion	Local glands	Lungs	Bronchial glands
221	Pus from knee	8	6th	177	14	sub.	988	1,690	1,870	K 100	Cystic	Nil	Small tubercles on surface	Nil
224	Abscess of hip	7	8th	181	11	sub.	25	2,200	2,170	K 100	Caseous	Nil	A few translucent foci	Nil
	" "	7	8th	181	11	sub.	26	2,250	2,420	K 100	Cystic	Cystic	Semi-confident tubercles on surface and at margins	Nil
225	Bone from hand	7	6th	144	13	sub.	29	2,370	2,340	K 100	Cystic	Nil	Several tubercles on surface	Nil
	" "	7	6th	144	13	sub.	30	1,800	1,790	K 100	Cystic	Nil	Surface and margins partially caseous	Nil
226	Tissue from knee	7	7th	154	11	sub.	21	1,820	1,850	K 100	Caseous	Nil	Extensive superficial caseation	Nil
	" "	7	7th	154	11	sub.	22	2,020	2,100	K 100	Caseous	Nil	A few tubercles	Nil
227	Pus from hip	8	10th	186	15	sub.	51	2,100	2,300	K 100	Caseous	Nil	A few small tubercles	Nil
	" "	8	10th	186	15	sub.	52	2,070	2,250	K 100	Cystic	Nil	Several small tubercles	Nil
228	Pus from hip	10	6th	173	11	sub.	991	2,100	2,120	K 102	Cystic	Cystic	Small grey tubercles on surface	Nil
	" "	8	9th	163	15	sub.	45	1,920	2,320	K 100	Caseous	Nil	A few tubercles on surface	Nil
	" "	8	9th	163	15	sub.	46	1,900	2,070	K 100	Caseous	Nil	A few small tubercles on surface	Nil
230	Psoas abscess	8	7th	178	14	sub.	39	2,300	2,550	K 100	Cystic	Caseous focus	A few small tubercles on surface	Nil
	" "	8	7th	178	14	sub.	40	1,800	1,950	K 100	Caseous	Nil	Numerous small tubercles on surface	Nil
231	Psoas abscess	8	9th	177	15	sub.	35	1,990	2,400	K 100	Cystic	Nil	Two translucent foci	Nil
	" "	8	9th	177	15	sub.	36	1,770	2,020	K 100	Cystic	Cystic	A few translucent foci	Nil
232	Pus " from hip	14	9th	186	13	sub.	47	1,940	2,000	K 100	Caseous	Nil	Slight marginal caseation	Nil
	" "	14	9th	186	13	sub.	48	2,300	2,350	K 100	Cystic	Nil	Nil	Nil
233	Psoas abscess	7	6th	146	13	sub.	2	1,650	1,820	K 102	Cystic	Nil	One caseous nodule and a few translucent foci	Nil

Kidneys  
Three, small  
tubercles

Spleen  
Nil

Several tubercles,  
some projecting,  
and scars on  
surface

Pits on surface  
A few minute  
tubercles

One minute focus

One small  
tubercle

Nil

Nil

Two small  
tubercles

Two small  
tubercles

Nil

Nil

Two small  
tubercles

Nil

Nil

Two small  
tubercles

Nil

Nil



233	Psoas abscess	7	6th	146	12	sub.	3	1,470	1,950	K 102	Caseous	Caseous focus	Nil	Nil	Nil	Nil
237	Pus from hip	14	6th	173	15	sub.	41	1,770	1,950	K 100	Caseous	Nil	Small caseous patches on surface	Nil	Nil	One minute focus
	"	14	6th	173	15	sub.	42	1,770	1,620	K 100	Caseous ulcer	Nil	A few small tubercles	Nil	Nil	Nil
238	Psoas abscess	14	8th	170	12	sub.	43	2,520	2,370	K 100	Cystic	Nil	Small grey tubercles on surface	Nil	Nil	Nil
	"	14	8th	170	12	sub.	44	2,650	2,900	K 100	Cystic	Nil	Small grey tubercles on surface	Nil	Nil	Nil
239	Bone from scapula	14	6th	139	12	sub.	50	1,920	2,050	K 101	Cystic	Nil	A few small translucent tubercles	Nil	Nil	Nil
	"	15	6th	140	12	sub.	53	1,870	1,900	K 100	Caseous	Caseous focus	Two translucent tubercles	Nil	Nil	Nil
240	Psoas abscess	8	8th	163	11	sub.	37	2,490	1,920	K 100	Caseous	Caseous	Thin caseous layer on two-thirds of surface	Nil	Nil	A few tubercles
	"	8	8th	163	11	sub.	38	2,170	2,340	K 100	Cystic	Caseous focus	Small grey tubercles numerous on surface	Nil	Nil	Several tubercles
241	Pus from knee	7	7th	178	12	sub.	81	1,140	2,170	K 101	Caseous	Nil	A few tubercles	Nil	Nil	One tubercle
243	"	7	7th	178	12	sub.	82	1,570	2,020	K 101	Caseous	Cystic	A few small tubercles	Cystic	Nil	Nil
243	Fluid from knee	6	7th	149	11	sub.	66	2,400	2,370	K 100	Caseous	Nil	Extensive superficial caseation	Nil	Nil	Nil
	"	6	7th	149	11	sub.	67	1,720	1,880	K 100	Caseous	Nil	Extensive superficial caseation	Nil	Nil	One tubercle
244	Scrapings from hip	7	6th	97	15	sub.	935	1,620	1,850	K 103	Cystic	Caseous foci	A few small tubercles	Nil	Nil	Nil
	"	7	6th	97	15	sub.	936	2,190	2,200	K 103	Caseous	Nil	Some grey tubercles on surface	Nil	Nil	Nil
246	Pus from hip	6	5th	70	11	sub.	915	1,850	1,590	D 93	Caseous	Caseous focus	Enlarged and replaced by caseous tissue	Caseous focus	Nil	Nil
	"	6	5th	70	11	sub.	916	2,120	1,790	D 84	Cystic	Caseous focus	Extensively replaced by caseous tissue	Nil	Nil	One small tubercle
	"	4	10th	157	15	sub.	131	1,870	1,800	K 101	Caseous	Caseous	A few small tubercles	Nil	Nil	One scar on surface
	"	4	10th	157	15	sub.	132	2,670	2,400	K 101	Caseous	Caseous	Small caseous tubercles	Nil	Nil	A few small tubercles
	"	4	3rd	32	10	sub.	129	1,640	2,050	K 101	Cystic	Nil	A few small tubercles	Nil	Nil	One scar on surface
	"	4	3rd	32	1	i.v.	130	1,670	1,540	K 101	—	—	Almost completely replaced by caseous tissue	Nil	Nil	Projecting tubercles on surface

TABLE III. *Rabbits inoculated with Viruses of "human" type—(concluded).*

No. of case	Source of cultures	Details of cultures				Mode of inoculation	No. of Rabbit	Weights of Rabbits in grammes		Duration of Experiment (days)	Post-mortem Results						
		Age of sub-culture in days	Generation	Total duration of incubation in days	Dose (mg.)			Initial	Final		Local lesion	Local glands	Lungs	Bronchial glands	Spleen	Kidneys	
246	Rabbit 130 (kidney)	7	4th	53	20	sub.	175	1,770	2,070	K 124	Caseous focus	Caseous	Nil	Discrete tubercles on surface and a few in substance	Nil	Nil	Nil
	" "	7	4th	53	20	sub.	176	1,670	1,500	K 124	Cystic	Caseous focus	Caseous	A few small tubercles on surface	Nil	Nil	Nil
247	Psoas abscess	7	5th	124	12	sub.	54	2,250	2,400	K 101	Cystic	Nil	Nil	A few translucent tubercles	Nil	Nil	One tubercle
	" "	7	5th	124	12	sub.	55	1,800	1,670	D 73	Cystic	Nil	Nil	A few superficial tubercles	Nil	Nil	Nil
248	Abscess of spine	6	6th	173	12	sub.	70	1,770	2,050	K 100	Caseous	Cystic	Nil	A few translucent foci	Nil	Nil	One grey focus
	" "	6	6th	173	12	sub.	71	1,670	1,790	K 100	Cystic	Nil	Nil	A few grey tubercles	Nil	Nil	One minute focus
249	Dressing from lumbar sinus	6	6th	140	12	sub.	91	2,150	2,120	K 104	Caseous	Caseous	Caseous	A few grey tubercles	Nil	Nil	One grey focus
	" "	6	6th	140	12	sub.	92	2,350	2,050	K 104	Caseous	Caseous	Caseous	A few grey areas on surface and at margins	Nil	Nil	A few small tubercles
251	Bone from ankle	17	5th	128	15	sub.	33	2,470	2,500	K 100	Cystic	Cystic	Nil	Nil	Nil	Nil	Nil
	" "	17	5th	128	15	sub.	34	2,170	2,150	K 100	Caseous	Nil	Nil	Nil	Nil	Nil	Pus in pelvis of both kidneys
252	Psoas abscess	10	5th	132	13	sub.	68	1,800	1,950	K 100	Cystic	Nil	Nil	A few grey tubercles	Nil	Nil	Nil
	" "	10	5th	132	13	sub.	69	2,080	1,950	K 100	Caseous	Cystic	Caseous	Small grey tubercles on surface	One small tubercle	One scar and one grey focus on surface	One grey tubercle
253	Pus from sternum	7	8th	162	12	sub.	58	2,200	2,200	K 101	Cystic	Cystic	Caseous	Small grey tubercles on surface	Nil	Nil	One grey tubercle
	" "	7	8th	162	12	sub.	59	1,750	1,900	K 101	Caseous	Caseous	Caseous	Partially replaced by caseous tissue	Nil	Nil	Nil
254	Bone from spine	6	7th	119	13	sub.	72	2,020	2,090	K 100	Cystic	Cystic	Caseous	A few translucent foci	Nil	Nil	Nil
	" "	6	7th	119	13	sub.	73	2,240	2,440	K 100	Cystic	Cystic	Caseous	A few grey tubercles	Caseous focus	Nil	Nil
256	Fluid from knee	5	10th	177	14	sub.	96	2,100	2,200	K 103	Cystic	Nil	Nil	A few small tubercles on surface	Nil	Nil	A few small tubercles
	" "	5	10th	177	14	sub.	97	2,000	2,270	K 103	Cystic	Nil	Nil	A few small tubercles on surface	Nil	Nil	A few scars on surface

257	Metatarsal bone	6	5th	120	12	sub.	87	2,350	2,420	K 104	Caseous	Caseous	A few yellow nodules and grey tubercles	Nil	Nil	Nil
	"	6	5th	120	12	sub.	88	2,700	1,800	K 104	Caseous ulcer	Nil	Thin caseous layer on parts of surface	Nil	Nil	Two scars on surface
259	Pus from hip	7	8th	160	15	sub.	79	1,520	1,900	K 101	Caseous ulcer	Caseous	Nil	Nil	Nil	Nil
260	"	7	8th	160	15	sub.	80	1,500	1,800	K 101	Cystic	Cystic	A few translucent foci	Nil	Nil	Nil
	Pus from spine	17	5th	140	15	sub.	31	2,270	2,350	K 100	Cystic	Nil	A few translucent foci and very slight marginal caseation	Nil	Nil	A few minute foci
	"	17	5th	140	15	sub.	32	2,190	2,100	K 100	Caseous	Cystic	Small grey tubercles numerous on surface	Nil	Nil	A few small tubercles
261	Pus from hip	9	8th	97	14	sub.	27	2,020	2,150	K 100	Caseous	Nil	A few small translucent areas on surface	Nil	Nil	Nil
263	"	9	8th	97	14	sub.	28	2,400	2,100	K 100	Caseous ulcer	Nil	A few translucent foci	Nil	Nil	Nil
	Tarsal bone	10	6th	128	15	sub.	989	2,340	2,620	K 100	Caseous	Caseous focus	A few small tubercles	Caseous	Nil	Nil
	"	10	6th	128	15	sub.	990	1,990	1,820	K 100	Cystic	Nil	Scattered tubercles on surface	Nil	Nil	One tubercle
264	Tissue from ear	6	9th	104	13	sub.	64	2,770	2,740	K 100	Caseous	Cystic	Nil	Nil	Nil	Nil
	"	6	9th	104	13	sub.	65	2,070	2,250	K 100	Caseous	Caseous focus	Two small tubercles	Nil	Nil	Nil
265	Tissue from rib	7	8th	147	15	sub.	83	1,800	1,940	K 101	Caseous	Nil	Small tubercles on surface and slight marginal caseation	Nil	Nil	Nil
	"	7	8th	147	15	sub.	84	1,850	1,770	K 101	Cystic	Cystic	Several grey tubercles on surface	Nil	Nil	Nil
266	Pus from hip	7	7th	139	10	sub.	60	2,220	2,400	K 101	Caseous	Caseous focus	Partial superficial caseation	Nil	Nil	Numerous scars on surface
	"	7	7th	139	10	sub.	61	1,800	2,070	K 101	Caseous	Caseous focus	Partially replaced by caseous tissue	Nil	Nil	Nil

days after subcutaneous inoculation with virus 21, showed generalised peritoneal tuberculosis, complete tuberculous solidification of the lungs, and advanced renal tuberculosis of bovine type. A culture from the kidney was dysgonic. As will be seen from the table, the companion rabbit, 618, developed only slight disease. The second and third rabbits showed advanced, generalised tuberculosis of intestinal origin. The former died 62 days after subcutaneous inoculation with virus 132, and yielded a dysgonic culture; the latter was killed 104 days after subcutaneous inoculation with virus 164. In both cases the companion animals, recorded in the table, showed only slight disease.

#### CHARACTERISTICS OF THE TUBERCLE BACILLI OF BOVINE TYPE.

##### *Cultures.*

The tubercle bacilli in this group were identical with bacilli of bovine origin in their growth on glycerinated media. The majority of the viruses were markedly dysgonic, in the usually accepted sense of that term. Whilst a few others yielded better growths, none grew so profusely as the "human" strains.

##### *Virulence for Experimental Animals.*

All the viruses were highly virulent for guinea-pigs and produced typical generalised tuberculosis in these animals.

Each virus was inoculated subcutaneously into at least two rabbits, in a dose of 10 mg. of culture (see Table IV, pp. 296-300). When both animals died in less than 100 days from typical generalised tuberculosis, we considered that the bovine type of the virus was definitely established. This was the case with 43 out of the 55 viruses. The remaining 12 called for some further investigation.

With regard to these 12, it will be observed from the table that, in every instance, (1) one rabbit (or more) died in less than 100 days with typical generalised tuberculosis of organs and lymphatic glands, but (2) one or more companion rabbits inoculated with culture of the same virus showed somewhat less severe infection. Cultures recovered after a single passage through a rabbit usually proved to have regained full virulence when tested in another series of rabbits. Whilst recognising that these differences in the results of inoculation may be attributed to variations in susceptibility of the rabbits, it must be



pointed out that similar differences rarely occur in rabbits inoculated with the same quantity of bacilli derived from the ox.

These experiments therefore show that the twelve viruses in question were appreciably, though only slightly, below the standard of "bovine" virulence for the rabbit. The age of the patients from which these viruses were derived ranged from 5 to 22 years and averaged 9.9 years. The age of the patients from which the 13 fully virulent viruses were obtained varied from two months to 17 years and averaged 6.2 years.

Four rabbits are omitted from Table IV. They all died prematurely from intercurrent disease in from 21 to 39 days after inoculation. In each case the amount of disseminated tuberculosis found at the time of death was compatible with the "bovine" character of the virus.

#### CHARACTERISTICS OF THE ATYPICAL TUBERCLE BACILLI.

Whilst we have found no difficulty in classifying all the preceding viruses as belonging either to the human or the bovine type, there remain the following, which differ from each of these types as regards the inter-relationship between cultural characters and virulence. In the present investigation we have found 10 such strains; to our records of these we have added notes on three others, which we met with in enquiries previously reported.

Of these 13 strains, six are indistinguishable culturally from bovine bacilli, viz. viruses 29, 32, and 262 obtained from bone and joint tuberculosis and viruses H 99, P LXXI, and P LXXVII discovered in two previous enquiries. But for rabbits the virulence of these six is that of the eugonic human bacillus.

The remaining seven strains are also of low virulence for the rabbit, but culturally they differ from both the human and the bovine type in one or other respect. They grow well on bovine serum, generally producing bright yellow pigment on suitable batches of serum; on glycerin-agar the growth is extremely slow and is in the form of very numerous, almost microscopic colonies, upon which a few large, convoluted, pigmented colonies ultimately supervene; on glycerin-potato the growth is slow but prolonged, resulting in the formation of large, peculiarly shaped, pigmented colonies. Growth on glycerin-broth has occurred only in the form of a few isolated islands.

The atypical characters of the above 13 viruses have been found to be permanent features and have not been altered by passage through rabbits.

TABLE IV. *Rabbits inoculated with "Bovine" Viruses.*

No. of case	Source of Cultures	Details of Cultures				Dose (mg.)	Mode of Inoculation	No. of Rabbit	Weights of Rabbits in grammes		Duration of Experiment (days)	Post-mortem Results
		Age of sub-culture in days	Generation	Total duration of culture in days					Initial	Final		
19	Psoas abscess	13	6th	107		10	sub.	472	1,620	1,200	D 31	General tuberculosis of organs and lymphatic glands.
	"	13	6th	107		10	sub.	473	1,500	1,120	D 55	"
23	Lumbar abscess	10	4th	65		10	sub.	513	2,290	2,000	K 101	Chronic general tuberculosis of organs and glands, but lungs mainly crepitant, with patches of early caseation.
	"	10	4th	65		10	sub.	514	1,720	1,070	D 81	General tuberculosis of organs and lymphatic glands.
	"	8	14th	204		.01	i.v.	750	1,550	1,300	D 61	"
	"	8	14th	204		10	sub.	752	1,600	—	K 100	"
	"	8	14th	204		10	sub.	753	1,950	1,190	D 96	Caseous ulcer at site of inoculation; caseous foci in both subscapular, right popliteal, and left inguinal glands; a few translucent foci and small grey patches on surface of lungs; all other tissues normal.
	"	8	14th	204		10	sub.	753	1,950	1,190	D 96	Chronic general tuberculosis, but inguinal and popliteal glands not affected; lungs not enlarged, but extensively caseous, with cavity formation; renal tubercles apparently retrogressive.
	"	8	14th	204		10	sub.	753	1,950	1,190	D 96	General tuberculosis of organs and lymphatic glands.
35	Rabbit 514 (kidney)	8	6th	83		10	sub.	850	1,620	1,420	D 45	"
	"	8	6th	83		10	sub.	851	2,050	1,400	D 56	"
	"	11	5th	62		10	sub.	502	2,500	1,800	D 43	"
	"	11	5th	62		10	sub.	503	2,150	1,600	D 32	"
36	Pus from mastoid	11	5th	62		10	sub.	448	1,460	900	D 76	"
	"	11	3rd	53		10	sub.	496	3,300	2,550	D 30	"
	"	11	6th	88		10	sub.	497	1,900	1,090	D 83	"
	"	11	6th	88		10	sub.	497	1,900	1,090	D 83	"
49	Psoas abscess	8	5th	46		10	sub.	525	2,540	1,700	D 40	"
	"	8	5th	46		10	sub.	526	2,040	1,500	D 42	"
	"	10	6th	83		10	sub.	627	2,550	1,650	D 82	"
54	Scraping from sinus of ankle	10	6th	83		10	sub.	627	2,550	1,650	D 82	"
	"	10	6th	83		10	sub.	628	1,400	870	D 37	"
55.	Psoas abscess	8	5th	75		10	sub.	669	1,700	1,400	D 53	"
	"	8	5th	75		10	sub.	670	2,170	1,520	D 59	"
56	Dressing from hip	7	6th	92		10	sub.	685	1,970	1,050	D 84	"
	"	7	6th	92		10	sub.	686	1,770	1,270	D 27	"
64	Lumbar abscess	11	4th	60		10	sub.	492	3,600	2,500	D 47	"
	"	11	4th	60		10	sub.	493	2,320	1,620	D 53	"
68	Pus from hip	4	5th	62		10	sub.	515	2,470	1,540	D 56	"
	"	4	5th	62		10	sub.	516	1,600	1,020	D 85	"

71	Pus from hip	6	5th	70	10	sub.	537	1,950	1,500	D 35	General tuberculosis of organs and lymphatic glands.
72	" " "	6	5th	70	10	sub.	538	1,950	1,400	D 53	" "
72	Pus from hip	11	5th	57	10	sub.	498	2,600	1,650	D 33	" "
	" " "	11	5th	57	10	sub.	499	1,950	1,240	K 101	Chronic general tuberculosis of organs and lymphatic glands.
73	Pus from hip	7	10th	225	10	sub.	829	1,800	1,050	D 80	General tuberculosis of organs and lymphatic glands.
	" " "	11	5th	56	10	sub.	494	3,600	2,600	D 84	Caseous ulcer at site of inoculation; left axillary gland caseous; all other lymphatic glands normal; spleen normal; lungs enlarged and completely replaced by caseous tissue; numerous tubercles in kidneys.
	" " "	11	5th	56	10	sub.	495	2,150	1,570	D 30	General tuberculosis of organs, but lungs only partially replaced by caseous tissue.
	" " "	5	12th	181	10	sub.	796	1,770	1,400	K 100	Caseous ulcer at site of inoculation; general tuberculosis of lymphatic glands; lungs not much enlarged, but mainly replaced by caseous tissue; spleen normal; a few minute tubercles in kidneys and depressions on surface.
78	Rabbit 494 (lung)	5	12th	181	10	sub.	797	1,540	950	D 39	General tuberculosis of organs and lymphatic glands.
	" " "	8	4th	58	10	sub.	754	1,750	1,020	D 93	" "
	" " "	8	4th	58	10	sub.	755	1,750	970	D 95	General tuberculosis of organs and lymphatic glands.
	Psoas abscess	5	6th	89	10	sub.	561	2,000	1,420	D 47	" "
	" " "	5	6th	89	10	sub.	562	1,300	1,100	D 53	" "
81	Psoas abscess	8	12th	147	10	sub.	667	1,370	1,120	D 42	" "
	" " "	8	12th	147	10	sub.	668	1,600	990	K 100	Caseous local lesion; caseation of one subcapsular and one bronchial gland, all other lymphatic glands normal; lungs not much enlarged but mainly replaced by caseous tissue, with cavity formation; spleen normal; tuberculous nodules in kidneys and testes.
	" " "	10	16th	273	10	sub.	913	2,050	1,570	K 100	Caseous local ulcer; subcapsular and bronchial glands caseous, inguinal and popliteal normal; lungs extensively replaced by caseous tissue; spleen normal; several tubercles in kidneys.
	" " "	10	16th	273	10	sub.	914	2,570	1,900	D 55	General tuberculosis of organs and lymphatic glands.
	Rabbit 668 (kidney)	5	5th	53	10	sub.	94	1,850	1,650	D 56	" "
	" " "	5	5th	53	10	sub.	95	1,800	1,450	D 75	" "
87	Psoas abscess	7	7th	129	10	sub.	663	2,300	1,550	D 77	" "
	" " "	7	7th	129	10	sub.	664	1,390	970	D 48	" "
89	Psoas abscess	7	10th	124	10	sub.	665	2,490	1,820	D 46	" "
	" " "	7	10th	124	10	sub.	666	1,750	1,170	D 36	" "
92	Psoas abscess	6	9th	113	10	sub.	715	2,120	1,530	D 45	" "
	" " "	6	9th	113	10	sub.	716	1,750	1,190	D 38	" "
94	Pus from hip	7	8th	102	10	sub.	671	1,740	1,220	D 43	" "
	" " "	7	8th	102	10	sub.	672	1,980	1,220	D 62	" "
102	Dressing from psoas abscess	11	9th	108	10	sub.	730	2,530	1,350	D 78	General tuberculosis of organs and glands, but lungs not completely replaced by caseous tissue.
	" " "	11	9th	108	10	sub.	731	2,010	1,290	D 81	General tuberculosis of organs and lymphatic glands.
	Rabbit 730 (popliteal gland)	18	4th	58	10	sub.	74	2,120	1,650	D 35	" "
	" " "	18	4th	58	10	sub.	75	1,600	1,150	D 62	Caseous local lesion; general tuberculosis of lymphatic glands; lungs almost completely replaced by caseous tissue; spleen normal; a few tubercles in kidneys.

TABLE IV. *Rabbits inoculated with "Bovine" Viruses—(continued).*

No. of case	Source of Cultures	Details of Cultures				Dose (mg.)	Mode of Inoculation	No. of Rabbit	Weights of Rabbits in grammes		Duration of Experiments (days)	Post-mortem Results
		Age of sub-culture in days	Generation	Total duration of culture in days	Initial				Final			
104	Dressing from psoas sinus	9	7th	82	10	sub.	675	1,820	1,350	D 59	General tuberculousis of organs and lymphatic glands.	
	"	9	7th	82	10	sub.	676	1,740	1,320	D 95	"	
	"	9	7th	79	10	sub.	673	2,270	1,380	D 52	"	
	Pus from empyaema	9	7th	79	10	sub.	674	2,000	1,170	D 70	"	
106	"	9	7th	90	10	sub.	677	2,090	1,450	D 45	"	
	Dressing from hip	9	7th	90	10	sub.	678	1,640	1,170	D 59	"	
107	"	9	6th	86	10	sub.	679	2,270	1,720	K 100	"	
	Dressing from hip	9	6th	86	10	sub.	679	2,270	1,720	K 100	Caseous ulcer at site of inoculation; caseation of peripheral glands; lungs largely replaced by caseous tissue, with cavity formation; spleen normal; in kidneys numerous nodules, pus in pelvis of one and pits on surface.	
	"	9	6th	86	10	sub.	680	1,850	1,100	D 66	General tuberculousis of organs and lymphatic glands.	
	"	10	7th	213	10	sub.	933	1,950	1,520	D 54	"	
	"	10	7th	213	10	sub.	934	1,700	1,100	K 103	Caseous ulcer at site of inoculation; general tuberculousis of lymphatic glands; lungs enlarged and completely replaced by caseous tissue; numerous large tubercles in kidneys; a few small tubercles in spleen.	
108	Dressing from psoas abscess	16	6th	92	10	sub.	681	2,100	1,500	D 33	General tuberculousis of organs and lymphatic glands.	
	"	5	11th	141	10	sub.	800	2,420	1,720	D 86	Caseous ulcer at site of inoculation; peripheral glands caseous; bronchial glands and spleen normal; lungs partially replaced by caseous tissue; a dozen small tubercles in kidneys.	
	"	5	11th	141	10	sub.	801	1,520	—	K 100	General tuberculousis of organs and glands, but a little crepitant tissue in lungs.	
	"	10	16th	262	10	sub.	117	2,200	1,440	D 93	Caseous ulcer at site of inoculation; caseation of subscapular glands and a few caseous foci in bronchial glands, but inguinal and popliteal glands normal; network of caseous tissue on surface of lungs and interior congested; a few tubercles on surface of spleen but none in substance; small tubercles in cortices of kidneys and scars on surface; many tubercles in walls of intestines.	
111	Pus from knee	7	8th	100	10	sub.	683	1,570	1,300	D 48	General tuberculousis of organs and lymphatic glands.	
	"	7	8th	100	10	sub.	684	1,430	1,040	D 78	"	
117	Dressing from spine	6	10th	120	10	sub.	776	1,520	1,170	D 59	"	
	"	6	10th	120	10	sub.	777	1,600	1,000	D 81	"	
133	Pus from hip	8	9th	151	10	sub.	770	1,750	1,170	D 79	"	
	"	8	9th	151	10	sub.	771	1,980	1,520	D 44	"	
135	" from hip	7	7th	144	10	sub.	748	1,430	1,050	D 64	"	
	"	7	7th	144	10	sub.	749	1,450	1,150	D 46	"	



136	Gluteal abscess	8	9th	150	10	sub.	762	1,530	1,320	D 65	
	"	8	9th	150	10	sub.	763	1,400	1,200	D 48	"
	Abscess of zygoma	8	6th	136	10	sub.	760	1,530	1,090	D 57	"
	"	8	6th	136	10	sub.	761	1,220	1,040	D 49	"
149	Psoas abscess	6	9th	134	10	sub.	772	1,700	1,150	D 98	"
	"	6	9th	134	10	sub.	773	1,870	1,150	D 70	"
155	Tendon sheath of hand	5	8th	137	10	sub.	798	1,750	1,370	D 62	"
	"	5	8th	137	10	sub.	799	1,400	990	D 42	"
160	Psoas abscess	8	7th	121	10	sub.	756	1,650	970	D 83	"
	"	8	7th	121	10	sub.	757	1,390	970	D 49	"
174	Dressing from temporal bone	7	5th	76	10	sub.	746	2,500	1,190	D 95	"
	"	7	5th	76	10	sub.	747	1,670	1,050	D 30	"
175	Dorsal abscess	8	6th	115	10	sub.	774	2,320	1,670	D 36	"
	"	8	6th	115	10	sub.	775	1,670	1,000	D 88	"
193	Dressing from hip	10	7th	133	10	sub.	899	2,090	1,150	D 75	"
	"	10	7th	133	10	sub.	900	2,470	1,500	D 57	"
194	Dressing from ankle	8	5th	79	10	sub.	764	1,740	1,520	K 100	"
	"	8	5th	79	10	sub.	765	2,170	1,150	D 57	"
	Rabbit 764 (kidney)	9	3rd	39	10	sub.	107	2,440	1,420	D 36	"
	"	9	3rd	39	10	sub.	108	1,870	1,450	D 30	"
197	Pus from hip	10	7th	119	10	sub.	802	1,000	1,500	K 100	"
	"	10	7th	119	10	sub.	803	2,070	1,100	D 93	"
	"	8	9th	232	15	sub.	85	2,010	1,070	D 74	"
	"	8	9th	232	15	sub.	86	2,100	1,340	D 57	"
	Rabbit 803 (kidney)	5	3rd	62	10	sub.	103	2,050	1,670	D 33	"
	"	5	3rd	62	10	sub.	104	1,920	1,120	D 67	"
198	Dressing from psoas sinus	9	6th	69	10	sub.	883	1,690	1,370	D 31	"
	"	9	6th	69	10	sub.	884	2,070	1,450	D 46	"
205	Bone from wrist	8	6th	104	10	sub.	768	1,920	1,150	D 55	"
	"	8	6th	104	10	sub.	769	2,120	1,500	D 92	"
	"	5	9th	249	10	sub.	113	2,470	1,600	D 76	"
	"	5	9th	249	10	sub.	114	2,350	1,700	D 45	"
206	Dorsal abscess	7	7th	126	10	sub.	895	1,140	1,000	D 45	"
	"	7	7th	126	10	sub.	896	1,690	1,240	D 31	"
210	Psoas abscess	10	6th	96	10	sub.	780	2,350	1,260	D 54	"
	"	10	6th	96	10	sub.	781	1,500	1,000	K 100	"

TABLE IV. *Rabbits inoculated with "Bovine" Viruses—(concluded).*

Details of Cultures											
No. of case	Source of Cultures	Age of sub-culture in days	(Generation)	Total duration in days	Dose (mg.)	Mode of inoculation	No. of Rabbit	Weights of Rabbits in grammes		Duration of Experiment (days)	Post-mortem Results
								Initial	Final		
210	Psoas abscess	12	11th	228	10	sub.	100	1,500	1,350	D 47	General tuberculosis of organs and lymphatic glands.
214	" "	12	11th	228	10	sub.	101	2,350	1,800	D 65	
214	Psoas abscess	8	6th	83	10	sub.	758	2,070	1,400	D 73	
215	" "	8	6th	83	10	sub.	759	1,480	1,100	D 86	
215	Pus from hip	8	4th	85	9	sub.	766	2,520	2,040	D 37	
220	" "	8	4th	85	10	sub.	767	1,600	1,300	D 45	
220	Pus from hip	9	6th	139	10	sub.	893	1,420	1,020	D 47	
222	" "	9	6th	139	10	sub.	894	2,270	1,650	D 48	
222	Pus from hip	7	7th	141	10	sub.	897	1,370	1,000	D 62	
223	" "	7	7th	141	10	sub.	898	1,780	1,350	D 69	
223	Psoas abscess	11	6th	96	10	sub.	826	1,850	1,500	D 42	
229	" "	11	6th	96	10	sub.	827	1,870	1,200	D 43	
229	Pus from hip	7	5th	86	10	sub.	806	1,670	1,220	D 38	
234	" "	7	5th	86	10	sub.	807	1,520	1,160	D 35	
234	Dressing from ankle	9	6th	104	10	sub.	885	2,690	1,400	D 43	
235	" "	9	6th	104	10	sub.	886	2,770	1,870	D 55	
235	Pus from hip	9	6th	99	10	sub.	881	1,870	1,500	D 49	
236	" "	9	6th	99	10	sub.	882	2,170	1,640	D 40	
236	Dorsal abscess	9	6th	118	10	sub.	889	1,650	1,070	D 82	
242	" "	9	6th	118	10	sub.	890	1,470	850	D 59	
242	Psoas abscess	9	5th	80	10	sub.	887	2,090	1,700	D 35	
245	" "	9	5th	80	10	sub.	888	1,700	1,220	D 46	
245	Psoas abscess	10	8th	129	10	sub.	909	2,250	1,420	D 77	
250	" "	10	8th	129	10	sub.	910	1,950	1,300	D 72	
250	Tissue from ankle	9	4th	69	10	sub.	891	1,720	1,240	K 100	
Caseous local ulcer; general tuberculosis of lymphatic glands; lungs enlarged and extensively replaced by caseous tissue; a few tubercles in spleen; caseous but not projecting tubercles and streaks in kidneys, with some small depressions on surface.											
General tuberculosis of organs and lymphatic glands.											
255	" "	9	4th	69	10	sub.	892	1,300	1,150	D 35	General tuberculosis of organs and lymphatic glands.
255	" "	7	7th	178	10	sub.	123	2,100	1,570	D 49	
255	" "	7	7th	178	10	sub.	124	1,920	1,350	D 32	
255	Rabbit 891 (lung)	4	3rd	37	10	sub.	127	2,420	1,650	D 43	
255	" "	4	3rd	37	10	sub.	128	2,020	1,570	D 51	
255	Pus from hip	10	6th	79	10	sub.	911	2,220	1,240	D 46	
258	" "	10	6th	79	10	sub.	912	1,970	1,440	D 56	
258	Metacarpal bone	6	5th	73	10	sub.	917	1,670	970	D 56	
258	" "	6	5th	73	10	sub.	918	2,200	1,650	D 29	
258	" "	6	5th	73	10	sub.				D 29	

The following is a detailed account of the characteristics of these viruses. Their pathogenicity for rabbits is set out in Table V.

*Virus 29.*

From psoas abscess of boy, aged five years, through guinea-pig.

Tested on culture media up to the 15th generation. No pigmentation observed in serum cultures<sup>1</sup>. On glycerin-agar a quickly growing, translucent, finely wrinkled, moist layer; sometimes small warty colonies developed subsequently; the growth always remained inferior to the eugonic "human" bacillus; on glycerin-potato a thin layer of growth, beset with discrete colonies.

General tuberculosis produced in guinea-pigs inoculated with original material. Slight virulence for four rabbits (Table V, p. 306) killed 100 days after inoculation. A fifth rabbit died from intercurrent disease 53 days after inoculation and showed only slight tuberculosis. A sixth rabbit, not included in the table, died from intercurrent disease in 22 days, with only slight evidence of tuberculosis.

*Virus 32.*

Direct culture from spinal caries of girl, aged 10 years.

Tested on culture media up to the 24th generation. No pigmentation observed in serum cultures. On glycerin-serum a moist, greasy layer, rather better than on pure serum. On glycerin-agar similar to Virus 29. On glycerin-potato a scanty growth, consisting of discrete, warty colonies.

Two guinea-pigs were inoculated intraperitoneally, each with .1 mg. of culture; they died from general tuberculosis in 74 and 75 days. Two, also, were inoculated subcutaneously, each with 1 mg. of culture. The first died in 153 days. There was no visible local lesion, and, with the exception of purulent foci in both inguinal glands and one sternal gland, no tuberculosis of lymphatic glands; there were grey fibroid areas in the liver and spleen; the lungs were normal. The second guinea-pig died from chronic fibroid tuberculosis in 224 days. Virulence was slight for six rabbits.

*Virus 34.*

Direct culture from sacral abscess of man, aged 50 years.

Tested on culture media up to 19th generation. No pigmentation observed in serum cultures. Growth on glycerin-serum a uniform

<sup>1</sup> Subsequently, A. S. Griffith, making an independent investigation of the same virus, has obtained pigmentation on serum.

layer inferior to that on pure serum. On glycerin-agar after several weeks a thin layer composed of numerous microscopical colonies, upon which there was subsequent and slow development of a few large, opaque, convoluted, pinkish colonies. On glycerin-potato growth slow and persistent, giving rise to large colonies resembling the cast of earthworms. Growth dysgonic on broth, in form of delicate grey islands.

Virulence of cultures high for guinea-pigs; low for rabbits and not raised by passage through a second series of rabbits. The characters of the cultures were unaltered by passage through rabbits. In addition to the nine rabbits shown in the table, one rabbit died from diarrhoea 42 days after intravenous inoculation with 0.1 mg.; there was only slight tuberculosis of lungs and kidneys.

*Virus 42.*

Direct cultures from (1) sacro-iliac abscess and (2) lumbar abscess of woman, aged 40 years.

Cultural characters of both strains identical. Thick growth on serum with marked yellow pigmentation. Growth on glycerin-serum similar to that on pure serum. On glycerinated agar, potato, and broth, identical with Virus 34.

Virulence of both strains identical; high for guinea-pigs, low for rabbits, and not increased by passage through rabbit. No change in cultural characters by passage through rabbits.

*Virus 67.*

Direct culture from psoas abscess of man, aged 36 years.

Tested on culture media up to 18th generation. Identical in every detail with cultures of Virus 42.

Virulence high for guinea-pigs, low for rabbits, and not increased by passage in the rabbit. In addition to the seven rabbits in the table, a rabbit died from intercurrent disease, 17 days after inoculation; there was no tuberculosis beyond the glands nearest to the local lesion. No change in cultural characters by passage through rabbits.

*Virus 83.*

Culture from lumbar abscess of boy, aged five years, through guinea-pig.

Cultural characters identical in every respect with those of Viruses 67 and 42.

Fully virulent for guinea-pigs; of low virulence for rabbits.



*Virus 138.*

Culture from abscess of hip of boy, aged four years, through guinea-pig.

Tested on culture media up to the 14th generation. Thick growths on pure serum and glycerin-serum, the latter with yellow pigment. Very minute colonies on glycerin-agar slowly becoming confluent but not raised. No growth on glycerin-potato. Dysgonic on glycerin-broth, a small grey island alone being formed.

Fully virulent for guinea-pigs; of very low virulence for rabbits.

*Virus 183.*

Culture from ankle of boy, aged three years, through guinea-pig. The boy afterwards died from tuberculous meningitis.

Highly pigmented on serum. Delicate, confluent colonies on glycerin-agar, a few becoming subsequently large, convoluted and pinkish. Discrete, irregular colonies on glycerin-potato. Dysgonic on glycerin-broth, like Viruses 67 and 42.

General tuberculosis produced in guinea-pigs inoculated with original material and with culture. Slightly virulent for two rabbits.

*Virus 192.*

Culture from hip of boy, aged 12 years, through guinea-pig.

Tested on culture media up to the 11th generation. Markedly pigmented on serum. Delicate, confluent colonies on glycerin-agar, a few becoming raised, large, convoluted, and opaque. Raised, pigmented colonies on glycerin-potato. Dysgonic on glycerin-broth. Identical with Viruses 67 and 42.

The original material produced general tuberculosis in guinea-pigs. Slightly virulent for rabbits; virulence not increased by passage through rabbits.

*Virus 262.*

Culture from psoas abscess of girl, aged 12 years, through guinea-pig.

Tested on culture media up to the 10th generation. Not pigmented on serum. A typical dysgonic "bovine" growth on glycerin-agar. A few minute opaque colonies on glycerin-potato.

Of somewhat lower virulence for guinea-pigs than the ordinary human or bovine bacillus. The two animals inoculated subcutaneously, each with 1 mg. of culture, survived 128 and 135 days, respectively.

Only slight disease was produced in two rabbits inoculated subcutaneously and in one inoculated intravenously with the original culture. Generalised tuberculosis with death in 77 days was caused in a second rabbit intravenously inoculated, a companion to the former. Virulence was not increased by passage through rabbits.

Of cultures obtained from three of the above mentioned rabbits, the culture from the severely affected intravenous rabbit produced only slight disease in a second pair of rabbits. The characters of the cultures remained unaltered.

The above atypical viruses may be compared with the following three, which have already been recorded in our previously published reports on the incidence of tuberculosis in children and tuberculosis in swine.

*Virus H 99.*

Culture, through guinea-pig, from mesenteric glands (obtained Dec. 6th, 1912) of male child,  $9\frac{3}{4}$  years, showing, post-mortem, spinal caries, ascites, and tuberculosis of the mesenteric glands and spleen.

Culture retested, November, 1914, in 16th generation. No pigmentation on serum. A thin grey layer on glycerin-serum. On glycerin-agar, after several weeks delay, numerous minute, almost microscopic colonies. On glycerin-potato discrete, large, raised and contorted colonies.

General tuberculosis was produced in a guinea-pig inoculated with the original human material. Tested on seven rabbits, with slightly virulent results. No increase of virulence after passage in a rabbit.

*Virus P LXXI.*

Direct culture obtained from the submaxillary lymphatic glands (obtained April 19th, 1912) of a pig. A case of localised tuberculosis.

Culture retested, November, 1914, in 22nd generation. Grey growth on serum. Very poor growth on glycerin-agar, consisting of three opaque, raised, umbilicated, slightly pink colonies. A crop of minute, raised colonies on glycerin-potato.

General tuberculosis produced in guinea-pigs after inoculation with original material and culture. Only slight virulence for nine rabbits. No increase of virulence after passage in rabbits.

*Virus P LXXVII.*

Direct culture obtained from the submaxillary lymphatic glands (obtained May 15th, 1912) of a pig. A case of localised tuberculosis.

Culture retested, November, 1914, in 21st generation. No pigmentation on serum. On glycerin-agar numerous discrete colonies, afterwards becoming raised, opaque, and confluent. Opaque, raised colonies on glycerin-potato.

General tuberculosis produced in guinea-pigs, but duration of life longer than the average. Of relatively low virulence for 11 rabbits. No increase of virulence after passage in rabbits.

TABLE V. *Rabbits inoculated with Atypical Viruses.*

Details of Cultures																
No. of case	Source of cultures	Age of sub-culture in days	Generation	Total duration of culture in days	Dose (mg.)	Mode of inoculation	No. of Rabbit	Weights of rabbits in grammes		Duration of Experiment (days)	Post-mortem Results				Spleen	Kidneys
								Initial	Final		Local lesion	Local glands	Lungs	Bronchial glands		
29	Psoas abscess	5	4th	92	14	sub.	474	2,000	2,150	K 100	Cystic	Caseous foci	Caseous patches on surface	Nil	Nil	A few caseous nodules
	"	10	5th	166	10	sub.	625	1,520	1,120	D 53	Caseous focus	One translucent focus	Nil	Nil	Nil	
	"	10	5th	166	10	sub.	626	1,650	2,120	K 100	Cystic	Nil	Eight small nodules	Nil	Nil	Nil
	"	7	10th	236	-01	i.v.	722	2,380	2,570	K 100	—	—	A few tubercles and slight marginal caseation	Nil	Nil	One minute tubercle
32	"	7	10th	236	-01	i.v.	723	2,010	2,000	K 100	—	—	A few small tubercles	Nil	Nil	In one, a few tubercles and pus in pelvis
	Pus from spine	11	5th	92	10	sub.	500	2,700	2,440	K 101	Caseous	Caseous	A few tubercles	Nil	Nil	Three grey foci
	"	11	5th	92	10	sub.	501	2,100	2,240	K 101	Caseous	Caseous	A few translucent foci	Nil	Nil	A few scars on surface
	"	6	19th	294	17	sub.	877	1,600	1,650	K 100	Cystic	Caseous focus	Several translucent foci	Nil	Nil	One small tubercle
34	"	6	19th	294	17	sub.	878	1,670	1,670	K 100	Cystic	Nil	A few caseous tubercles and some marginal caseation	Nil	Nil	A few tubercles
	"	6	19th	294	-1	i.v.	879	1,900	1,320	K 100	—	—	Crepitant; numerous small, discrete tubercles	Caseous foci	A few minute tubercles	Numerous small caseous streaks
	"	6	19th	294	-1	i.v.	880	1,750	970	D 94	—	—	Not enlarged, but numerous small, discrete, caseous tubercles	Caseous foci	One yellow tubercle	Numerous tubercles and pus in pelvis
	"	6	19th	294	-1	i.v.	880	1,750	970	D 94	—	—	Not enlarged, but numerous small, discrete, caseous tubercles	Caseous foci	One yellow tubercle	Numerous tubercles and pus in pelvis
34	Sacral abscess	10	5th	45	10	sub.	430	1,660	—	D 85	Caseous	Caseous	Almost completely replaced by caseous tissue	Nil	A few grey foci	Nil
	"	10	5th	45	10	sub.	431	1,650	1,620	K 101	Cystic ulcer	Caseous	Small caseous nodules on surface	Nil	Nil	One tubercle
	"	8	9th	141	-01	i.v.	604	1,770	1,600	K 101	—	—	Some small tubercles	Nil	Nil	Nil
	"	8	9th	141	14	sub.	605	2,050	2,000	K 101	Cystic	Cystic	Several discrete tubercles	Nil	Nil	Nil
"	"	8	9th	141	15	sub.	606	1,920	1,800	K 101	Caseous	Caseous foci	Partial caseation of surface	Nil	Nil	Three small tubercles



34	Rabbit 430 (lung)	9	4th	51	10	sub.	659	1,660	1,570	K 100	Caseous ulcer	Caseous	Superficial caseation	Nil	Nil	One grey focus
	"	9	4th	51	15	sub.	660	2,180	1,440	D 75	Caseous	Cystic	Superficial caseous patches	Caseous foci	Nil	A few grey foci and streaks in cortices
	Rabbit 431 (scapular gland)	6	5th	63	11	sub.	697	1,490	1,520	K 100	Cystic	Nil	A few superficial caseous patches	Nil	Nil	Nil
	"	6	5th	63	11	sub.	698	1,620	1,500	K 100	Caseous ulcer	Caseous	Small caseous patches on surface	Nil	Nil	One grey focus
42	Sacro-iliac abscess	8	3rd	30	10	sub.	444	2,030	2,100	K 100	Caseous ulcer	Caseous	Largely replaced by caseous tissue	Nil	Nil	Nil
	"	8	3rd	30	10	sub.	445	1,330	1,400	K 100	Caseous ulcer	Caseous foci	A few small tubercles	Nil	Nil	Nil
	"	8	7th	115	-01	i.v.	607	2,100	1,970	K 101	—	—	A few small tubercles	Nil	Nil	Several small tubercles
	"	8	7th	115	20	sub.	608	1,670	1,850	K 101	Caseous	Nil	A few caseous patches	Nil	Nil	A few small tubercles
	"	8	7th	115	-01	i.v.	609	1,470	970	D 95	—	—	A few small tubercles	Nil	Nil	One grey focus
	"	8	7th	115	20	sub.	610	1,680	1,550	K 101	Caseous ulcer	Caseous foci	Surfaces nearly covered with thin caseous layer	Nil	Two minute foci	Numerous small tubercles
	Rabbit 444 (scapular gland)	6	4th	53	10	sub.	695	2,040	2,100	K 100	Cystic	Nil	A few translucent foci and slight marginal caseation	Nil	Nil	One small tubercle
	"	6	4th	53	10	sub.	696	1,630	1,470	K 100	Cystic	Nil	Caseation of surface; caseous nodules in substance, with cavity formation	Nil	Nil	Nil
	Lumbar abscess	7	5th	95	-01	i.v.	575	1,920	2,150	K 100	—	—	Slight patches of caseation	Nil	Nil	A few grey foci and scars on surface
	"	7	5th	95	-01	i.v.	576	1,650	1,550	K 100	—	—	Slight patches of caseation	Nil	One minute tubercle	Pus in pelvis of one; several tubercles
	"	7	5th	95	10	sub.	578	1,970	1,900	K 100	Cystic	Caseous	Many early tubercles on surface	Nil	Two grey foci	A few small tubercles
67	"	7	5th	95	10	sub.	579	1,590	1,800	K 100	Cystic	Cystic	A few translucent tubercles	Nil	Nil	Nil
	"	7	3rd	40	10	sub.	464	2,370	2,220	K 100	Cystic	Nil	Caseous nodules and patches on surface	Nil	Nil	Nil
	"	7	3rd	40	20	sub.	465	1,970	1,900	K 100	Cystic	Cystic	Caseous patches on surface	Nil	Nil	Pelvis of one caseous
	"	7	6th	102	-01	i.v.	569	1,400	2,100	K 100	—	—	Numerous early tubercles; bulk of tissue crepitant	Nil	Nil	Several small tubercles

TABLE V. *Rabbits inoculated with Atypical Viruses—(continued).*

No. of case	Source of Cultures	Details of Cultures				Mode of Inoculation	No. of Rabbits	Weights of Rabbits in grammes		Duration of Experiment (days)	Post-mortem Results				Spleen	Kidneys
		Age of sub-culture in days	(generation)	Total duration of culture in days	Dose (mg.)			Initial	Final		Local lesion	Local glands	Lungs	Bronchial glands		
67	Psoas abscess	7	6th	102	20	sub.	577	2,070	2,300	K 100	Cystic	Caseous	Several small tubercles	Nil	One caseous tubercle	One grey focus
	"	7	10th	187	-01	i.v.	724	2,500	2,450	K 100	—	—	Numerous soft, discrete nodules	Nil	Nil	Four caseous tubercles
	"	7	10th	187	-01	i.v.	725	1,950	2,040	K 100	—	—	Extensively replaced by caseous tissue	Nil	Nil	A few caseous tubercles
	Rabbit 465 (lung)	6	3rd	46	10	sub.	714	1,700	2,070	K 100	Cystic	Nil	Numerous discrete tubercles	Nil	Nil	In one, a few tubercles and pus in pelvis
83	Lumbar abscess	9	7th	118	10	sub.	653	2,650	2,200	K 100	Cystic	Caseous focus	Several small tubercles	Nil	Nil	Two minute tubercles
	"	9	7th	118	10	sub.	654	1,660	1,570	K 100	Caseous foci	Nil	A few small tubercles	Nil	Nil	Nil
	"	9	9th	177	-01	i.v.	792	1,990	1,550	K 100	—	—	Caseous patches on surface	Nil	Nil	Three tubercles
138	"	9	9th	177	-1	i.v.	793	2,050	1,520	K 100	—	—	Slight caseation of surface and margins	Nil	Nil	A few caseous tubercles and several scars on surface
	"	9	9th	177	15	sub.	794	1,110	1,090	K 100	Caseous	Nil	Thin caseous layer on surface	Nil	Nil	Nil
	"	9	9th	177	20	sub.	795	1,120	1,070	K 100	Cystic	Nil	Partial caseation of surface	Nil	Nil	Nil
183	Abscess of hip	7	5th	101	10	sub.	736	1,720	1,750	K 100	Caseous	Nil	Two small tubercles	Nil	Nil	Nil
	"	7	5th	101	10	sub.	737	1,740	1,950	K 100	Caseous	Nil	One minute tubercle	Nil	Nil	Nil
	"	7	5th	101	10	sub.	737	1,740	1,950	K 100	Caseous	Nil	Small tubercles on surface	Nil	Nil	Nil
192	Dressing from ankle	10	8th	182	15	sub.	927	2,070	2,220	K 103	Cystic	Nil	Small tubercles on surface	Nil	Nil	Nil
	"	10	8th	182	15	sub.	928	2,170	2,000	K 103	Cystic	Nil	Small tubercles on surface	Nil	Nil	Nil
	"	18	6th	143	16	sub.	907	2,370	2,020	K 100	Cystic	Caseous focus	Small semi-transparent tubercles	Nil	Several small tubercles	One tubercle in cortex; pus in pelvis of one
Rabbit 907 (kidney)	"	18	6th	143	16	sub.	908	2,150	2,240	K 100	Cystic	Nil	A few tubercles on surface	Nil	Nil	One grey focus
	"	8	6th	95	15	sub.	139	2,550	2,500	K 101	Caseous ulcer	Nil	Small caseous patches	Nil	Nil	Two small tubercles
	"	8	6th	95	30	sub.	140	2,200	2,100	K 101	Cystic	Nil	Small tubercles on surface	Nil	Nil	One minute tubercle

Psoas abscess	6	6th	77	10	sub.	931	1,670	1,370	K 103	Caseous	Caseous foci	Small gray tubercles on surface A few caseous patches on surface	Caseous foci	Nil	Several tubercles
"	6	6th	77	10	sub.	932	2,100	2,100	K 103	Cystic	Nil		Caseous	Nil	Nil
"	9	10th	126	01	i.v.	105	1,200	1,900	K 102	—	—	One caseous patch and several discrete tubercles on surface	Nil	Nil	A few small tubercles
"	9	10th	126	01	i.v.	106	2,030	1,500	D 77	—	Caseous	Almost completely replaced by caseous tissue	Caseous	Caseous	Abundant projecting tubercles
Rabbit 106 (popliteal gland)	8	6th	50	10	sub.	141	2,500	1,800	K 101	Caseous ulcer	Caseous	Scattered tubercles on surface and in substance	Nil	Nil	Numerous tubercles, projecting on surface
"	8	6th	50	10	sub.	142	2,150	1,720	K 101	Caseous	Caseous	Ramifying caseous areas on surface and in substance	Nil	Nil	Several tubercles and scars
Rabbit 931 (kidney)	16	5th	144	10	sub.	149	1,850	1,900	K 100	Caseous ulcer	Caseous	A few foci on surface	Nil	Nil	Nil
"	16	5th	144	10	sub.	150	1,880	1,800	K 100	Caseous	Caseous	A few small tubercles	Nil	A few small tubercles on surface	Small tubercles and depressions on surface
Rabbit 105 (kidney)	14	3rd	96	10	sub.	151	1,870	1,920	K 100	Caseous	Caseous	Numerous small tubercles, mostly superficial	Caseous foci	Nil	Small tubercles and depressed scars
"	14	3rd	96	10	sub.	152	1,620	1,700	K 100	Cystic	Caseous	Discrete tubercles on surface and at margins	Nil	Nil	A few small tubercles

## THE TYPES OF TUBERCLE BACILLI OCCURRING IN TUBERCULOSIS OF THE HUMAN GENITO- URINARY TRACT.

BY A. EASTWOOD, M.D., AND FRED GRIFFITH, M.B.

This report deals with the bacteriological characteristics of the tubercle bacilli isolated from seventeen<sup>1</sup> consecutive cases of tuberculosis affecting one or other part of the genito-urinary system.

For the supply of material we wish to express our indebtedness to the hospitals specified in the following table and to the many surgeons, physicians, and pathologists who have been kind enough to place both hospital and private cases at our disposal, and to provide us with clinical notes.

The methods of investigation were the same as those described in our previous reports. Cultures from urines were usually obtained through guinea-pigs inoculated with centrifuged deposit; in the case of two specimens the deposit was antiforminised and direct cultures were obtained.

### ADDITIONAL CLINICAL AND BACTERIOLOGICAL DATA.

Regarding the cases where the bacilli were found to be of "human" type, there is nothing to add to the clinical facts stated in Table I. In cultural characters the bacilli were all typically eugonic; this is in accordance with the results of the tests on rabbits (see Table II, below), which show that they were all of relatively low virulence for this animal.

The three cases where the bacilli proved to be "bovine" call for fuller description.

*Case 1.* E. H., female. First sample of urine received on May 1st, 1912, when patient was 24 years old. She suffered from frequency of micturition but her general health was good. In August, 1910, she had been examined in hospital under anaesthetic and material containing tubercle bacilli had been obtained from each kidney. Subsequent

<sup>1</sup> Six cases from which negative results were obtained are excluded from the series. In four of these negative cases egg tubes were inoculated with the spleens of guinea-pigs killed six weeks after intraperitoneal inoculation with centrifuged deposit of urine. In every instance the culture tubes remained sterile.



samples of urine were received for examination on August 22nd, 1912, December 9th, 1912, February 4th, 1913, and October 17th, 1913. Throughout this period her general condition remained as before. In 1914 she married and left London. At the end of that year her general health was still reported to be good.

Six cultures in all were obtained and tested, viz., direct cultures from the antiforminised deposits of the first and second specimens and cultures from guinea-pigs inoculated with the first, third, fourth and fifth specimens. All the cultures were found to be typically "bovine," being highly dysgonic on glycerinated media and of high virulence for rabbits. Cultures from the later specimens showed no indication of modification in the "human" direction, and, as will be seen from Table III, below, there was no abatement of their virulence for the rabbit.

*Case 2.* A. D., male, 19 years. Shortly after a specimen of urine had been received from this case, the left kidney was removed by operation and found to be extensively tuberculous.

The culture from the urine was highly dysgonic and typically "bovine" on glycerinated media. As will be seen from Table III, the tests on rabbits also justify the designation "bovine," though in two of the rabbits the pathogenic effects were less severe than those exhibited by the typical bacillus of bovine origin.

*Case 17.* R. K., female, 20 years. History of tuberculous cervical glands, of which the scars remained, in early childhood. Specimen of urine received on May 22nd, 1914. Symptoms of renal disease first occurred in August, 1913. The surgeon diagnosed tuberculosis of the right kidney. He informed us that she was improving under Beranek's tuberculin and vaccines prepared from time to time for the secondary infection present. There was no pyrexia except after an inoculation, and not much then.

Bacteriologically, the tubercle bacilli were identical with those from Case 1.

#### SUMMARY.

Seventeen cases were examined, the disease affecting the genital organs in nine instances (seven testes, one salpinx, one prostate) and the urinary tract in eight.

The bacilli obtained were of "human" type in fourteen cases and "bovine" in three.

The three "bovine" cases were affections of the kidney in persons aged, respectively, 25, 19 and 20 years.

TABLE I.  
*Clinical Data and Bacteriological Results.*

No. of Case	Initials of Patient	Sex	Age in years	Institution or Home Address	Situation of tuberculous lesions	Source of Cultures Isolated		Type of Tubercle Bacillus
						Original Material	Direct or through G.P.	
1	E. H.	F	24-5	(Private) London	Both kidneys	Urine	Both	Bovine
2	A. D.	M	19	St Bartholomew's Hosp., E.C.	Left kidney	"	Through G.P.	"
3	D. W.	M	30	St Peter's Hosp., W.C.	Right kidney, bladder, epididymis	"	"	Human
4	G. C.	M	38	"	Both kidneys	"	"	"
5	E. L.	F	20	St Bartholomew's Hosp., E.C.	Salpinx	Tissue from	"	"
6	W. E.	M	45	St Peter's Hosp., W.C.	Kidneys and bladder	Urine	"	"
7	V. B.	F	11	Fulham Inf., W.	Abdomen, lungs, ? right kidney	"	"	"
8	E. H.	F	19	(Private) Ipswich	Bladder	"	"	"
9	C. S.	M	15	St Bartholomew's Hosp., E.C.	Testicle	Epididymis	"	"
10	A. M.	M	20	"	"	"	Direct	"
11	M. J.	M	28	(Private) London	"	"	"	"
12	R. J.	M	52	Southern Hosp., Liverpool	"	"	"	"
13	D. N.	M	20	(Private) London	Prostate	Urine	Through G.P.	"
14	S. N.	M	34	St Bartholomew's Hosp., E.C.	Bladder	"	"	"
15	W. P.	M	22	(Private) Margate	Testicle	Epididymis	Direct	"
16	D. W.	M	34	St Bartholomew's Hosp., E.C.	"	Testicle	"	"
17	R. K.	F	20	(Private) Doncaster	Right kidney	Urine	Through G.P.	Bovine

TABLE II. *Rabbits inoculated with Eugonic Viruses.*

No. of Case	Source of Cultures	Age of sub culture in days	Generation	Total duration of culture in days	Dose (mg.)	Mode of Inoculation	No. of Rabbit	Weights of Rabbits in grammes		Duration of Experiments (days)	Post-mortem Results*						
								Initial	Final		Local lesion	Local glands	Lungs	Bronchial glands	Spleen	Kidneys	
3	Urine (through G.P.)	4	3rd	45	10	sub.	482	2,100	2,120	K 101	Cystic	Cystic	A few translucent tubercles	Nil	Nil	Nil	Two small tubercles
	" "		3rd	45	13	sub.	483	1,850	1,720	K 101	Cystic	Nil	Several discrete tubercles	Nil	Nil	Two small tubercles	
4	Urine (through G.P.)	8	6th	85	10	sub.	622	1,630	1,720	K 100	Caseous	Caseous	A few translucent tubercles	Nil	Nil	Nil	Two grey foci
	" "	7	7th	190	10	sub.	824	2,270	1,990	K 100	Caseous	Cystic	Few small caseous patches	Nil	Nil	Two grey foci	
5	" (through G.P.)	5	3rd	36	10	sub.	825	1,590	1,420	K 100	Cystic	Nil	Thin caseous layer on surface	Nil	Nil	Nil	
	Salpinx (through G.P.)	5	3rd	36	10	sub.	476	2,770	3,150	K 100	Cystic	Nil	A few caseous patches on surface	Nil	Nil	Nil	
	" "	6	5th	90	10	sub.	589	2,250	1,700	K 100	Cystic	Nil	A few translucent tubercles	Nil	Nil	Nil	
	" "	6	5th	90	10	sub.	590	1,650	1,870	K 100	Cystic	Nil	A few translucent tubercles	Nil	Nil	Nil	
6	Urine (through G.P.)	7	5th	68	13	sub.	571	2,020	2,140	K 100	Cystic	Caseous	Nil	Nil	Nil	Nil	
	" "	7	5th	68	13	sub.	572	1,300	1,190	K 100	Cystic	Nil	A few small tubercles	Nil	Nil	Nil	
7	Urine (through G.P.)	7	3rd	32	11	sub.	533	1,700	1,600	K 100	Cystic	Caseous	Thin caseous layer nearly covering surface	Nil	Nil	A few grey foci	
	" "	7	3rd	32	11	sub.	534	2,270	2,000	K 100	Cystic	Caseous	A few flat tubercles on surface	Nil	Nil	Nil	
8	Urine (through G.P.)	7	5th	104	10	sub.	740	1,950	2,050	K 100	Cystic	Nil	Translucent tubercles on surface	Nil	Nil	One tubercle	
	" "	7	5th	104	10	sub.	741	1,200	1,500	K 100	Cystic	Nil	Caseous tubercles and patches on surface	Caseous focus	Nil	A few small tubercles	
9	Epididymis (through G.P.)	9	6th	115	11	sub.	788	2,720	2,200	K 100	Cystic	Nil	A few grey patches on surface	Nil	Nil	One grey focus	
	" "	9	6th	115	11	sub.	789	1,700	1,620	K 100	Caseous	Nil	A few small tubercles on surface	Nil	Nil	One grey focus	
10	Epididymis (direct)	7	7th	144	11	sub.	743	3,080	3,050	K 100	Caseous	Nil	A few small tubercles	Nil	Nil	A few pits on surface	
	" "	10	10th	227	12	sub.	903	1,920	2,090	K 100	Cystic	Nil	A few small tubercles	Nil	Nil	One caseous focus	
	" "	10	10th	227	12	sub.	904	2,390	2,120	K 100	Cystic	Nil	A few small tubercles	Nil	Nil	One minute focus	
11	Epididymis (direct)	9	8th	151	14	sub.	786	1,910	1,650	K 100	Caseous	Caseous focus	Nil	Nil	Nil	Nil	
	" "	9	8th	151	14	sub.	787	1,850	1,700	K 100	Cystic	Nil	A few translucent foci	Nil	Nil	Nil	
12	Epididymis (direct)	11	7th	172	11	sub.	853	2,150	2,140	K 100	Caseous	Nil	A few translucent foci	Nil	Nil	One small tubercle	
	" "	11	7th	172	11	sub.	854	1,850	2,040	K 100	Caseous	Nil	A few translucent tubercles	Nil	Nil	Nil	
13	Urine (through G.P.)	6	7th	204	14	sub.	943	1,900	2,000	K 100	Cystic	Nil	Two small tubercles	Nil	Nil	Nil	
	" "	6	7th	204	14	sub.	944	2,020	2,200	K 100	Cystic	Nil	Two small tubercles	Nil	Nil	Nil	
14	Urine (through G.P.)	7	4th	162	11	sub.	963	1,450	1,620	K 100	Caseous	Cystic	Scattered tubercles on surface	Nil	Two small tubercles	Two small tubercles	
	" "	7	4th	162	11	sub.	964	1,550	1,640	K 100	Cystic	Nil	A few translucent tubercles	Nil	Two small tubercles	tubercles	
15	Epididymis (direct)	11	6th	170	13	sub.	5	1,970	1,890	K 101	Cystic	Caseous	Extensive superficial caseation	Nil	Nil	Several small tubercles	
	" "	11	6th	170	13	sub.	6	1,690	1,720	K 101	Cystic	Nil	Extensive superficial caseation	Nil	Nil	One grey tubercle	
16	Testicle (direct)	7	6th	157	14	sub.	56	1,920	2,120	K 101	Cystic	Nil	One translucent focus	Nil	One small tubercle	Two tubercles	
	" "	7	6th	157	14	sub.	57	1,750	1,800	K 101	Caseous	Caseous	A few translucent tubercles	Nil	Two tubercles	Nil	

\* In addition to the rabbits recorded in this table, three died prematurely from intercurrent disease, 44, 54 and 58 days after inoculation. Only a slight amount of tuberculosis was found in each case.

TABLE III  
*Rabbits inoculated with Dysgonic Viruses.*

No. of Case	Source of Cultures	Details of Cultures				Dose (mg.)	Mode of Inoculation	No. of Rabbit	Weights of Rabbits in grammes		Duration of Experiments (days)	Post-mortem Results	
		Age of sub-culture in days	Generation	Total duration of culture in days					Initial	Final			
1	1st spec. urine (through G.P.)	11	4th	51		·01	i.v.	82	2,200	1,970	D 35	General tuberculosis of organs and lymphatic glands.	
	1st spec. urine (direct)	9	5th	83		·01	i.v.	84	2,070	1,400	D 24		
	" "	9	5th	83		1	i.m.	85	2,020	1,640	D 34		
	2nd spec. " "	16	4th	74		10	sub.	122	2,180	1,400	D 22		
	" " "	16	4th	74		10	sub.	123	2,170	1,350	D 51		
2	3rd spec. urine (through G.P.)	10	4th	90		5	sub.	218	1,130	1,200	D 26	Chronic general tuberculosis; kidneys beset with tubercles projecting above surface; lungs not enlarged but contain numerous tubercles and caseous nodules; spleen and bronchial glands normal. Cystic local lesion; caseous foci in subcapsular, bronchial, and one popliteal gland; lungs partially replaced by caseous tissue; spleen normal; tubercles in kidneys; some projecting on surface, and pus pelvis of one.	
	4th spec. urine (through G.P.)	9	4th	90		10	sub.	351	2,400	1,750	D 63		
	5th spec. urine (through G.P.)	4	6th	74		10	sub.	519	2,800	2,100	D 37		
	" " "	6	6th	85		10	sub.	567	1,770	1,020	D 38		
	Urine (through G.P.)	6	6th	85		10	sub.	568	1,920	1,370	K 101		
17	" " "	10	11th	210		10	sub.	804	2,130	1,240	K 100	General tuberculosis of organs and lymphatic glands.	
	" " "	10	11th	210		10	sub.	805	1,550	890	D 61		
	Urine (through G.P.)	6	6th	76		10	sub.	929	2,200	1,870	D 35		
	" " "	6	6th	76		10	sub.	930	1,970	1,330	D 39		
	" " "												



## THE AUSTRALIAN EPIDEMIC, 1914.

BY A. B. GREEN, M.A., M.D., B.C. (CANTAB.).

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Lister Institute of Preventive Medicine.*

IN November, 1914, the Director of the Lister Institute sent me a sealed tube containing dried crusts or scabs which were stated to have been removed from patients, subjects of the Australian epidemic earlier in the year. Dr Martin requested me to investigate any ascertainable facts as to their pathological nature, and what relation, if any, they bore to vaccinia on the one hand and to variola on the other.

The clinical aspect of the epidemic has been described by Dr Armstrong<sup>1</sup>, while certain bacteriological investigations of the disease have been reported by Drs J. Burton-Cleland and E. W. Ferguson<sup>2</sup>.

Although the majority of the medical authorities in Australia are stated to have regarded the epidemic as one of small-pox, probably of a slight nature, doubt appears to have existed in the minds of some of the observers as to whether this was actually the case, and the present investigation is an outcome of this uncertainty.

The crusts or scabs had been forwarded to England in cold storage from Australia, and there were in all some 40 gms. of them. Throughout the time of the investigation the crusts were stored in a desiccator at a temperature of about 4° C., small quantities being removed from time to time for use as required. The material for the inoculation of animals was invariably composed as follows, one part of crusts ground up in a pestle and mortar with four times its own bulk of 50 per cent. glycerine and water, the mixture being made immediately before use. The variolar material used as a control was from a case of confluent small-pox in a man, and the specific activity of this was ascertained by inoculation on monkeys; it was strongly active throughout. The vaccine lymph

<sup>1</sup> *Proceedings of the Royal Society of Medicine, Section of Epidemiology and State Medicine*, vol. VIII. No. 2, p. 1.

<sup>2</sup> *Ibid.* p. 19.

used as another control was seed-lymph used for the production of lymph at these laboratories and was very active, as shown by frequent inoculations on calves.

## I.

The first series of animal experiments was made on guinea-pigs, in view of the fact that some observers had expressed the opinion that the Australian disease was a form of modified small-pox; and modification, if sufficiently extended, might give the material a vaccine character. Drs J. Burton-Cleland and E. W. Ferguson had found that the material which they employed gave typical vaccine vesicles when inoculated direct on bovines. As stated in a previous paper on vaccinia<sup>1</sup>, if a non-immune buck guinea-pig be vaccinated on the scrotum, vaccine vesicles develop with great facility about 72 hours later. The present experiments were made in a place remote from calf vaccine work, and the technique was arranged to prevent any infection being conveyed outside. 70 buck guinea-pigs were inoculated on the scrotum with material from the Australian disease. No trace either of vesiculation or of any reaction of any kind was subsequently noted, the small incisions healing normally. 70 control pigs, inoculated at the same time and in the same manner with calf vaccine, developed in each case typical vaccine vesicles 72 hours later.

This failure to react to the crusts is no proof that the cases from which the crusts were taken were not small-pox, but it is of evidential value in tending to show that whatever modification the disease may have undergone from ordinary small-pox in its transition to "mild" small-pox (Armstrong, Burton-Cleland, Ferguson), such transition had not carried it to vaccinia. Subsequent experiments showed that the failure of the material to react was not due to loss of its own specific activity.

## II.

The second series of inoculations was made on calves. The experiments on Calves 1-4 were made in the stables where routine calf vaccination is carried out, at the Lister Institute. Those on Calves 5 and 6 were made in a place remote from all such work, and the technique was arranged to obviate the possibility of any infection being conveyed away from the animals.

<sup>1</sup> Green. *Journal of Hygiene*, 1914.

CALF 1 (Heifer). (a) Was inoculated on a shaved area on the abdomen with the Australian disease. 120 hours later there were found along the lines of inoculation a series of yellow vesicles about the size of lentils; the appearance was unlike that of ordinary vaccine vesicles, being much smaller and more discrete. On the other hand their appearance was unlike that resulting from an inoculation of small-pox material direct from man to the calf, being, from my experience, too developed, even for a first inoculation positive result.

(b) At the same time a second area was inoculated with calf vaccine, and 120 hours later typical vaccine vesicles had developed on the site, these bearing practically no resemblance to the small yellow vesicles of the Australian disease.

CALF 2 (Bull). (a) Was inoculated on the shaved scrotum with the Australian disease. 120 hours later small yellow vesicles had developed as in the case of Calf 1.

(b) At the same time an area on the abdomen was inoculated with calf vaccine, and 120 hours later typical vaccine vesicles of first-class quality, and bearing no resemblance to the vesicles of the Australian disease, had developed here. The results on this calf resembled those on Calf 1.

CALF 3 (Heifer). (a) Was inoculated on a shaved area on the abdomen with vesicular contents removed from the Australian disease vesicles of Calf 1. The vesicular material had been removed at 120 hours after inoculation, and had been stored for two weeks at 4° C. 120 hours later the result on this site was *nil*.

(b) A second area was inoculated with similarly stored vesicular material from the Australian disease vesicles of Calf 2. 120 hours later vesicles had developed at the site, whose appearance was somewhat suggestive, but not convincing, of poor class vaccine vesicles. Passage through three subsequent calves failed to improve the quality of this vesicular material, *i.e.* to make it resemble vaccinia more closely clinically.

(c) A third area was inoculated with vaccine lymph and 120 hours later normal vaccine vesicles of good class had developed.

CALF 4. (a) A shaved area on the abdomen was inoculated with the stored material of the Australian disease vesicles of Calf 1. 120 hours later the result was *nil*.

(b) A second area on the abdomen was inoculated with stored material of the Australian disease vesicles from Calf 2. 120 hours

later vesiculation had resulted, the vesicles resembling somewhat poor class vaccine vesicles. Passage through two further calves failed to improve the quality of the vesicles.

(c) A third area was inoculated with vaccine lymph and 120 hours later normal vaccine vesicles of good class had developed.

CALF 5. Was inoculated with vesicles of the Australian disease removed from Monkey No. 2 (*vide* series of monkey experiments). Vesicles resembling moderately fair quality vaccine vesicles had developed 120 hours later.

CALF 6. Was inoculated with the Australian disease vesicles removed from Monkey No. 4. 120 hours later vesicles resembling vaccine vesicles of moderately fair quality had developed.

CALF 7. (a) A shaved area on the abdomen was inoculated with the Australian disease. 120 hours later there was a distinct reaction, with small papules and vesicles along the lines of inoculation, the result being similar to that of Calf 1, but not quite so marked. Here again the result was not typical of vaccinia, and it was too marked for variola inoculation direct from man.

(b) A fortnight later a second shaved area on the abdomen was inoculated with:

(1) Variolous material direct from a case of confluent small-pox in a man. 120 hours later the results were *nil*, no reaction of any kind being noticeable;

(2) Vaccine lymph. 120 hours later typical vaccine vesicles, but not of first-class quality, had developed.

CALF 8. (a) A shaved area on the abdomen was inoculated with:

(1) Vaccine. 120 hours later typical vaccine vesicles had developed.

(2) Variolous material from man, passed once through a monkey, on which it had given marked typical vesiculation. 120 hours later small vesicles had developed, not typical of vaccinia (but not so well developed as those of Australian disease); they approximated more to the Australian disease vesicles than to the vaccinal vesicles however.

(b) A fortnight later another area on the abdomen was shaved and inoculated with Australian crust emulsion. 120 hours later there was slight vesiculation at the site about the same appearance as on Calf 5, resulting from inoculation with Australian disease.

The results of the above experiments are more easily seen in Table I.

There are thus, with regard to these calf experiments, two main questions to consider:



1. *The likeness-relation of the Australian disease to Vaccinia and to Variola.*

The most prominent fact in all the calf experiments was that the vesiculation of the Australian disease, such as it was, was not generally typical of the vaccinia vesicles on the same calf, nor, I may be allowed to remark, was it typical of any vaccine vesicles that I remember on any calf. After passage through a monkey, however, the material gave rise to vesicles undistinguishable from those of moderately fair vaccine vesicles. The general impression arrived at was that the Australian disease, both by the appearance of the vesicles, and by their time-development, possessed some vaccino-variola relationship. Allowing that there were possibilities of such a relationship, the Australian disease would have to be classed as distinct on the one hand from vaccinia in its lesser ability to produce typical vaccinal vesiculation, though it approximated more closely after passage through a monkey; and on the other hand from variola, for without doubt it possessed a greater facility for vesiculating on a calf than did the variola in the above experiments.

In the foregoing experiments the vesicle-likeness was consistent, but it differed widely from the vesicle-likeness reported in the experiments by Drs Burton-Cleland and Ferguson. In the experiments by these observers there was a tendency for vesicles to develop very freely at any time, even in the case of repeated re-vaccinations at short intervals.

2. *The immunity-relation of the Australian disease to Vaccinia and to Variola.*

There are only two sets of data from which to draw deductions. Exps. 7 and 8 in Table I. This scarcity is due to two causes: (a) the comparative difficulty of obtaining calves at the present time, and maintaining them for a period necessary for the development of possible immunity and obtaining re-vaccination results; (b) the inferiority of the results obtained from inoculating calves with the Australian disease, and incidentally with variola—even when this had been passed through a monkey.

Taking these two sets of data for what they are worth, however, it appeared that successful vaccination with variola and vaccinia afforded no protection against subsequent inoculation with Australian disease, and the Australian disease gave no protection against vaccinia. The

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failure of variola to "take" on Calf 5 is of no account, as variolous material inoculated on the calf direct from man (*i.e.* not passed through a monkey) rarely gives definite vesiculation.

TABLE 1.

*Calf Experiments.*

Calf No.	Vaccination	Result	Vaccination	Result
1.	<i>A</i>	+		
		+		
	<i>L</i>	+		
		+		
2.	<i>A</i>	+		
		+		
	<i>L</i>	+		
		+		
		+		
		+		
3.	<i>A</i> (from Calf 1)	-		
	<i>L</i> (from Calf 2)	+		
	<i>L</i>	+		
		+		
		+		
4.	<i>A</i> (from Calf 1)	-		
	<i>A</i> (from Calf 2)	+		
	<i>L</i>	+		
		+		
		+		
5.	<i>A</i>	+		
	(from Monkey No. 2)	+		
		+		
		+		
6.	<i>A</i>	+		
	(from Monkey No. 4)	+		
		+		
7.	<i>A</i>	+	<i>V</i>	-
			<i>L</i>	+
				+
				+
				+
8.	<i>L</i>	+		
		+		
		+		
		+		
	<i>V</i>	+	<i>A</i>	+
	(Passed once through monkey)			

*A* = Australian disease, *L* = Calf lymph, *V* = Variola.

These two results, showing no protection, are in accord with the majority of the results of the calf experiments of Cleland and Ferguson. The accompanying Table II has been compiled for the sake of easier reference from the paper of Cleland and Ferguson<sup>1</sup>, which should of course be consulted for full information.

It will be seen that in three instances vaccination gave a positive result following repeated successful reactions with the Australian

TABLE II.

*Cleland and Ferguson's Calf Experiments.*

Calf No.	2/11	4 days later	21/10	4th day?	7/11	4th day?	24/11	4th day?	11/12	4th day?	30/12	4th day?
1.	A	+	L	+	A	+	A	-	A	+	L	+
				+								+
				+								
	16/10		7/11		24/11		11/12		30/12			
2.	A	+	A	+	A	-	A	+	L	+		
				+				+		+		
	7/11		24/11		11/12		30/12					
3.	A	+	A	+	A	+	L	+				
		+										
		+										
	11/12		30/12									
4.	A	+	L	-								
		+										
		+										
	24/11		17/12		9/1							
5.	L	+	L	-	A	+						
		+										
		+										
	15/1											
6.	A	+										
		+										
		+										
	15/11		22/1	9 days later								

*Monkey Experiments.*

1.	A	+	L	- ?
	22/1	9 days later		
2.	L	?		

A=Australian disease, L=calf lymph.

<sup>1</sup> *Proceedings of the Royal Society of Medicine, Section of Epidemiology and State Medicine*, Vol. VIII. No. 2, p. 1.

disease, and only one case a negative. In one case, Calf 5, the authors obtained a positive result of Australian inoculation following successful vaccination, *i.e.* complete protection was afforded only in 25 per cent. of the cases. In view of this fact it is difficult to understand Cleland and Ferguson's claim—"This inoculated disease and vaccinia are mutually more or less completely protective against each other provided sufficient area is inoculated." It would seem, indeed, that the number of Cleland and Ferguson's experiments is far too small to permit of the attempt to select one of the five (the sixth was not, of course, a cross immunity experiment at all) as establishing a law. The only deduction that it would seem safe to draw is the obvious one before mentioned, that 75 per cent. of the cases of inoculated Australian disease failed to protect against subsequent vaccination.

As before stated, the results of the experiments on calves in the present series of experiments were not such as to allow one to place much reliance on them in any way, and for this reason the series of experiments was not extended, extension preferably being made with monkey experiments, which gave very much better inoculation results.

### III.

The third series of experiments was made on rhesus and bonnet monkeys. The work was done in a place remote from all calf vaccine work, in order that no question of any accidental inoculation could arise, and under conditions of strict isolation to prevent the possibility of any infection being conveyed away from the monkeys. Each animal during the experimental period was kept in a separate cage by itself. Each animal was inoculated on an area of shaved skin over the scapulae, in order to reduce to a minimum the prospect of the results being spoilt by scratching. Each shaved area was about 6 square cm., and two linear incisions were made close together, allowing sufficient room for subsequent inoculations.

MONKEY 1. *Inoculation.* 1. xii. 14. Vaccinia.

*Result.* Typical vaccination vesicles of rather poor quality.

*Inoculation.* 12. i. 15. Australian disease.

*Result.* Definite vesiculation undistinguishable from results of calf vaccine of poor quality.

*Conclusion.* Here a successful vaccination with vaccinia afforded no protection against the subsequent inoculation of Australian disease.



MONKEY 2. *Inoculation.* 1. xii. 14. Australian disease.

*Result.* Well developed vesicles, resembling typical vaccine vesicles of good quality. Subsequent inoculation on Calf 5 gave vesiculation resembling that of calf vaccine.

*Inoculation.* 12. i. 15. Vaccinia.

*Result.* 19. i. 15. Full well developed vesicles of "good" quality.

*Inoculation.* 9. ii. 15. On one area vaccinia, and on another Australian disease.

*Result.* 16. ii. 15. Nil in each case.

*Conclusion.* This experiment suggested that while Australian disease failed to protect against vaccinia, the original inoculations of vaccine and Australian disease afforded protection against subsequent inoculation of the virus of these diseases—lymph against lymph and Australian disease against Australian disease.

MONKEY 3. *Inoculation.* 1. xii. 14. Australian disease.

*Result.* 8. xii. 14. Definite vesiculation, but tendency for discrete vesiculation to form along the lines of incision and for vesicles to be irregular.

*Inoculation.* 12. i. 15. Vaccinia.

*Result.* 19. i. 15. Typical vesiculation with tendency to irregularity.

*Conclusion.* There was manifestly no protection afforded here by the Australian disease against vaccinia.

MONKEY 4. *Inoculation.* 8. xii. 14. Australian disease.

*Result.* 15. xii. 14. Well developed vesicles undistinguishable from vaccine vesicles of good quality. This vesicular material inoculated on Calf 6 gave vesicles resembling vaccine vesicles of fair quality.

*Inoculation.* 12. i. 15. Vaccinia.

*Result.* 19. i. 15. Typical vesiculation of good quality.

*Inoculation.* 9. ii. 15. Australian disease on one area, vaccinia on another.

*Result.* 16. ii. 15. Nil in each case.

*Conclusion.* The experiment would appear to indicate that Australian disease failed to protect against subsequent vaccinia, but that these two diseases protected against a further subsequent double inoculation on separate areas of the same diseases.

MONKEY 5. *Inoculation.* 9. ii. 15. Australian disease.

*Result.* 16. ii. 15. Rather poor vesiculation.

*Inoculation.* 2. iii. 15. Variola.

*Result.* 9. iii. 15. *Nil.*

*Conclusion.* Australian disease possibly protected against variola.

MONKEY 6. *Inoculation.* 9. ii. 15. Australian disease.

*Result.* 16. ii. 15. Good vesiculation.

*Inoculation.* 2. iii. 15. Vaccinia on one area, variola on the other.

*Result.* 9. iii. 15. Vaccinia doubtful, variola good vesiculation.

*Conclusion.* Australian disease did not protect against variola, and probably not against vaccinia.

MONKEY 7. *Inoculation.* 9. ii. 15. Vaccinia.

*Result.* 16. ii. 15. Good vesiculation.

*Inoculation.* 2. iii. 15. Vaccinia on one area, variola on another.

*Result.* 9. iii. 15. Vaccinia *nil*, variola *nil*.

*Inoculation.* 23. iii. 15. Australian disease.

*Result.* 30. iii. 15. Slight papulation.

*Conclusion.* Vaccinia protected against vaccinia and variola, but not against Australian disease.

MONKEY 8. Died before any cross immunisation could be attempted.

MONKEY 9. Died before any cross immunisation could be attempted.

MONKEY 10. *Inoculation.* 9. ii. 15. Variola.

*Result.* 16. ii. 15. Fairly good vesiculation.

*Inoculation.* 2. iii. 15. Australian disease on one area, vaccinia on another.

*Result.* 9. iii. 15. Australian disease good vesiculation, vaccinia *nil*.

*Conclusion.* Variola protected against vaccinia, but not against Australian disease.

MONKEY 11. *Inoculation.* 16. ii. 15. Variola.

*Result.* 23. ii. 15. Fair vesiculation.

*Inoculation.* 2. iii. 15. Australian disease on one area, vaccinia on another.

*Result.* 9. iii. 15. Australian disease definite but slight vesiculation, vaccinia *nil*.

*Conclusion.* Variola protected against vaccinia, but not against Australian disease, or only to a very moderate degree.

MONKEY 12. *Inoculation.* 23. ii. 15. Mixed aerobic and anaerobic growth, cocci in broth, cultivated from Australian disease crusts.

*Result.* 2. iii. 15. *Nil.*

*Inoculation.* 2. iii. 15. Australian disease.

*Result.* 9. iii. 15. Definite vesiculation.

*Inoculation.* 23. iii. 15. Vaccinia on one area, variola on another.

*Result.* 30. iii. 15. Vaccinia fair vesiculation, variola *nil*.

*Conclusion.* (1) Cocci cultivated from crusts not pathogenic.  
(2) Australian disease did not protect against vaccinia, but may have protected against variola.

MONKEY 13. *Inoculation.* 2. iii. 15. Vaccinia on one area, variola on another.

*Result.* 9. iii. 15. Vaccinia fair vesiculation, variola strongly marked vesiculation.

*Inoculation.* 23. iii. 15. Australian disease.

*Result.* 30. iii. 15. Fair vesiculation.

*Conclusion.* Vaccinia and variola have not protected against Australian disease.

MONKEY 14. *Inoculation.* 2. iii. 15. Australian disease on one area, variola on another.

*Result.* 9. iii. 15. Australian disease fair vesiculation, variola poor vesiculation.

*Inoculation.* 23. iii. 15. Vaccinia.

*Result.* 30. iii. 15. Good vesiculation.

*Conclusion.* Australian disease does not protect against vaccinia, but neither did variola protect against vaccinia; it must be noted that the variola gave poor result.

MONKEY 15. *Inoculation.* 10. iii. 15. Variola.

*Result.* 17. iii. 15. Good vesiculation.

*Inoculation.* 23. iii. 15. Australian disease on one area, vaccinia on another.

*Result.* 30. iii. 15. Australian disease fair vesiculation, vaccinia *nil*.

*Conclusion.* Variola protected against vaccinia, but not against Australian disease.

MONKEY 16. *Inoculation.* 10. iii. 15. Variola.

*Result.* 17. iii. 15. Very marked vesiculation.

*Inoculation.* 23. iii. 15. Australian disease on one area, vaccinia on another.

*Result.* 30. iii. 15. Australian disease *nil*, vaccinia poor vesiculation.

*Conclusion.* Variola protected against Australian disease, and not against vaccinia, or only very slightly.

MONKEY 17. *Inoculation.* 10. iii. 15. Variola.

*Result.* 17. iii. 15. Rather poor vesiculation.

*Inoculation.* 23. iii. 15. Australian disease on one area, vaccinia on another.

*Result.* 30. iii. 15. Australian disease *nil*, vaccinia poor vesiculation.

*Conclusion.* Variola protected against Australian disease, but not against vaccinia, or only partially.

MONKEY 18. *Inoculation.* 10. iii. 15. Australian disease.

*Result.* 17. iii. 15. Vesiculation.

*Inoculation.* 23. iii. 15. Vaccine on one area, variola on another.

*Result.* 30. iii. 15. Vaccine first-class vesicles, variola fairly good vesicles.

*Conclusion.* Australian disease gave no protection against vaccine or variola.

MONKEY 19. *Inoculation.* 10. iii. 15. Australian disease.

*Result.* 17. iii. 15. Vesiculation fair.

*Inoculation.* 23. iii. 15. Vaccinia on one area, variola on another.

*Result.* 30. iii. 15. Poor vesiculation in each case.

*Conclusion.* Australian disease afforded no protection against vaccinia or variola, or only partial protection.

MONKEY 20. *Inoculation.* 10. iii. 15. Australian disease.

*Result.* 17. iii. 15. Vesiculation.

*Inoculation.* 23. iii. 15. Vaccinia on one area, variola on another.

*Result.* 30. iii. 15. Vaccinia good vesicles, variola very good.

*Conclusion.* Australian disease had afforded no protection against vaccinia or variola.

MONKEY 21. *Inoculation.* 10. iii. 15. Vaccinia.

*Result.* 17. iii. 15. Fair vesiculation.

*Inoculation.* 23. iii. 15. Australian disease on one area, variola on another.

*Result.* 30. iii. 15. Australian vesiculation good, variola *nil*.

*Conclusion.* Vaccinia afforded protection against variola, but not against Australian disease.

MONKEY 22. *Inoculation.* 10. iii. 15. Vaccinia.

*Result.* 17. iii. 15. Fair vesiculation.

*Inoculation.* 23. iii. 15. Australian disease on one area, variola on another.

*Result.* 30. iii. 15. Australian disease *nil*, variola *nil*.



*Conclusion.* Vaccinia protected against variola, and may have protected against Australian disease; at any rate the Australian disease failed to develop after previous successful vaccination.

The foregoing results have been condensed and tabulated in Table III.

TABLE III.

Monkey No.	Inoculation and Date	Result	Inoculation and Date	Result	Inoculation and Date	Result
1.	1/12/14 <i>L</i>	8/12/14 +	12/1/15 <i>A</i>	19/1/15 + Died		
2.	1/12/14 <i>A</i>	8/12/14 + + +	12/1/15 <i>L</i>	19/1/15 + + +	9/2/15 <i>L</i> : <i>A</i>	16/2/15 - : - Died
3.	1/12/14 <i>A</i>	8/12/14 + +	12/1/15 <i>L</i>	19/1/15 + + Died		
4.	8/12/14 <i>A</i>	15/12/14 + + +	12/1/15 <i>L</i>	19/1/15 + + +	9/2/15 <i>L</i> : <i>A</i>	16/2/15 - : - Died
5.	9/2/15 <i>A</i>	16/2/15 +	2/3/15 <i>V</i>	9/3/15 -		
6.	9/2/15 <i>A</i>	16/2/15 + +	2/3/15 <i>V</i> : <i>L</i>	9/3/15 + : ? Died		
7.	9/2/15 <i>L</i>	16/2/15 + +	2/3/15 <i>V</i> : <i>L</i>	9/3/15 - : - Died	23/3/15 <i>A</i>	30/3/15 +
8.	9/2/15 <i>L</i>	16/2/15 + Died				
9.	9/2/15 <i>V</i>	16/2/15 + Died				
10.	9/2/15 <i>V</i>	16/2/15 + +	2/3/15 <i>A</i> : <i>L</i>	9/3/15 + : - Died		
11.	16/2/15 <i>V</i>	23/2/15 +	2/3/15 <i>A</i> : <i>L</i>	9/3/15 + : - Died		
12.	23/2/15 Cocci from <i>A</i>	2/3/15 -	2/3/15 <i>A</i>	9/3/15 +	23/3/15 <i>L</i> : <i>V</i>	30/3/15 + : - Died
13.	2/3/15 <i>L</i> : <i>V</i>	9/3/15 + : + Died	23/3/15 <i>A</i>	30/3/15 +		
14.	2/3/15 <i>A</i> : <i>V</i>	9/3/15 + : + Died	23/3/15 <i>L</i>	30/3/15 + +		
15.	10/3/15 <i>V</i>	17/3/15 + +	23/3/15 <i>A</i> : <i>L</i>	30/3/15 + : - Died		

TABLE III—(continued).

Monkey No.	Inoculation and Date	Result	Inoculation and Date	Result
	10/3/15	17/3/15	23/3/15	30/3/15
16.	V	+	A : L	- : +
		+		
		+		
		+		
17	10/3/15	17/3/15	23/3/15	30/3/15
	V	+	A : L	- : +
	10/3/15	17/3/15	23/3/15	30/3/15
18.	A	+	L : V	+ : +
				+
				+
				+
19.	10/3/15	17/3/15	23/3/15	30/3/15
	A	+	B : V	+ : +
	10/3/15	17/3/15	23/3/15	30/3/15
20.	A	+	L : V	+ : +
				+
				+
				+
21.	10/3/15	17/3/15	23/3/15	30/3/15
	L	+	A : V	+ : -
22.	10/3/15	17/3/15	23/3/15	30/3/15
	L	+	A : V	- : -

V=variola, L=calf lymph, A=Australian disease.

As in the case of the calf experiments it will be convenient to consider this series of monkey experiments in two main aspects.

1. *The likeness-relation of the Australian disease to Vaccinia and to Variola.*

In this series (as has been previously remarked in the general statement) there is little or none of the ambiguity attaching to the question that appeared in the case of the calf experiments. Without exception the Australian disease vesicles resembled typical vaccine vesicles so closely that it was impossible to distinguish one condition from the other. In time-relation too the two appeared identical, there was indeed no apparent clinical distinction.

2. *The immunity-relation of the Australian disease to Vaccinia and to Variola.*

For the clearer consideration of this problem the experiments have been divided into sub-series in the following four tables (Tables IV, V, VI and VII).

*In the first sub-series (Table IV).* Those experiments have been collected in which monkeys were in the first place inoculated with

TABLE IV.

(Sub-series 1.)

Monkey No.	Inoculation and Date	Result	Inoculation and Date	Result	Inoculation and Date	Result
	1/12/14	8/12/14	12/1/15	19/1/15	9/2/15	16/2/15
2.	A	+	L	+	L : A	-
		+		+		Died
	1/12/14	8/12/14	12/1/15	9/2/15		
3.	A	+	L	+		
		+		Died		
	8/12/14	15/12/14	12/1/15	19/1/15	9/2/15	16/2/15
4.	A	+	L	+	L : A	-
		+		+		
5.	9/2/15	16/2/15	2/3/15	9/3/15		
	A	+	V	-		
6.	9/2/15	16/2/15	2/3/15	9/3/15		
	A	+	L : V	+		
		+		+		
	10/3/15	17/3/15	23/3/15	30/3/15		
18.	A	+	L : V	+		
				+		
				+		
	10/3/15	17/3/15	23/3/15	30/3/15		
19.	A	+	L : V	+		
	10/3/15	17/3/15	23/3/15	30/3/15		
20.	A	+	L : V	+		
				+		
				+		
				+		

V=variola, L=calf lymph, A=Australian disease.

TABLE V.

(Sub-series 2.)

Monkey No.	Inoculation and Date	Result	Inoculation and Date	Result	Inoculation and Date	Result
	1/12/14	8/12/14	12/1/15	19/1/15		
1.	L	+	A	+		
				Died		
	9/2/15	16/2/15	2/3/15	9/3/15	23/3/15	30/3/15
7.	L	+	L : V	-	A	+
		+				
		+				
21.	10/3/15	17/3/15	23/3/15	30/3/15		
	L	+	A : V	+		
		+		-		
22.	10/3/15	17/3/15	23/3/15	30/3/15		
	L	+	A : V	-		
		+				

A=Australian disease, V=variola, L=calf lymph.

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TABLE VI.

*(Sub-series 3.)*

Monkey No.	Inoculation and Date	Result	Inoculation and Date	Result
10.	9/2/15	16/2/15	2/3/15	9/3/15
	V	+	A : L	+ : -
		+		
11.	16/2/15	23/2/15	2/3/15	9/3/15
	V	+	A : L	+ : -
		+		
15.	10/3/15	17/3/15	23/3/15	30/3/15
	V	+	A : L	+ : -
		+		
16	10/3/15	17/3/15	23/3/15	30/3/15
		+		
	V	+	A : L	- : +
		+		
17.	10/3/15	17/3/15	23/3/15	30/3/15
	V	+	A : L	- : +
		+		

A=Australian disease, V=variola, L=calf lymph.

TABLE VII.

*(Sub-series 4.)*

Monkey No.	Inoculation and Date	Result	Inoculation and Date	Result
12.	2/3/15	9/3/15	23/3/15	30/3/15
	A : L	+ : +	V	-
13.	2/3/15	9/3/15	23/3/15	30/3/15
		+ : +		+
	L : V	+ : +	A	+
14.	2/3/15	9/3/15	23/3/15	30/3/15
		+ : +		+
	A : V	+ : +	L	+
				+

A=Australian disease, V=variola, L=calf lymph.

Australian disease and subsequently with vaccinia and variola—one after the other, or simultaneously.

*In the second sub-series (Table V).* Those experiments have been collected in which monkeys were in the first place inoculated with vaccinia, and subsequently with Australian disease and variola—one after the other, or simultaneously.

*In the third sub-series (Table VI).* Those experiments have been collected in which monkeys were in the first place inoculated with variola, and subsequently with Australian disease and vaccinia—in every case simultaneously.



*In the fourth sub-series (Table VII).* Those experiments, only three in number, have been collected which do not conform with those of the previous groups, and consist of initial double inoculations, with subsequent cross immunisation inoculations, as shown in the Table.

*In sub-series 1.* The eight initial inoculations of Australian disease, as shown in Table IV, were all successful in yielding vesicles, and some of the vesicles were of noticeably good quality, and well developed. Of these there were three cases of subsequent inoculation with vaccinia alone which all yielded vesicles, one case of subsequent inoculation with variola, which gave no reaction, and four cases of subsequent inoculation with vaccinia and variola simultaneously, which all gave definite typical vesiculation. In the whole eight cases therefore of this sub-series there was only one case (Monkey 5) to suggest that Australian disease afforded protection against vaccinia or variola. On the other hand the remaining seven cases indicate that no protection whatever had been afforded by Australian disease against subsequent vaccinia or variola. It only seems possible therefore to state that the percentage of immunity afforded by Australian disease in this sub-section was *nil* or very small.

That these animals were capable of developing immunity is shown in the cases of Monkeys 2 and 4, where re-inoculations of Australian disease and vaccinia respectively gave negative results.

*In sub-series 2 (Table V).* Consisting of four experiments, all initial inoculations with vaccinia gave typical vesiculation. All four animals were subsequently inoculated with Australian disease, and in three instances this was followed by vesiculation typical of vaccinia vesicles; the remaining one failed to react—a result of 75 per cent. against, and 25 per cent. for protection.

*In sub-series 3 (Table VI).* The five monkeys were all primarily inoculated with variola and gave vesiculation undistinguishable from the vesicles of vaccinia and Australian disease. Each case was subsequently inoculated with Australian disease and vaccinia simultaneously; in three cases the Australian inoculation was followed by typical vesiculation and in two cases by no reaction, indicative of conferred immunity in 40 per cent. and absence of conferred immunity in 60 per cent. If we take the cases of sub-series 2 and 3 together, vaccinia or variola may have conferred immunity in 33·3 per cent. cases, and did not confer it in 66·6 per cent. It must be noted however, that in sub-series 3 the possible protection afforded against Australian disease occurred in the same percentage as the protection afforded by variola against vaccinia.

*In sub-series 4 (Table VII).* In Monkey No. 12, Australian disease or vaccinia protected against subsequent inoculations with variola; in Monkey No. 13, vaccinia and variola failed to protect against Australian disease; and in Monkey No. 14 Australian disease and variola failed to protect against vaccinia.

Taking the four sub-series as a whole, evidence as to the ability of Australian disease to protect against vaccinia or variola was small or absent, but vaccinia or variola possibly protected against Australian disease in 30 per cent. of the cases; vaccinia protected against variola in 100 per cent. of the cases, and variola protected against vaccinia in 50 per cent. of the cases.

In comparing the foregoing percentage of vaccinia and variola protection one against the other, it is important to remember that the variola was inoculated experimentally, *i.e.* the infective material gained both a more intimate contact with the tissues than would be the case in usual variola infection, and was applied in much larger doses. The same point must be noted in connection with the Australian disease, and the fact that protection was afforded against variola and not against Australian disease, has the more significance, inasmuch as it occurred under this more stringent application of infection.

It may be noted in the foregoing text that when vaccinia or variola has failed to develop after previous successful inoculation with Australian disease the fact has been stated as such; but when variola has failed to develop after previous successful vaccination it is definitely assumed that this is in consequence of the vaccination. This variation of expression is owing to the circumstance that it is an accepted fact that vaccinia protected against variola, but it is not yet an accepted fact that Australian disease protects against vaccinia or variola.

Briefly stated, the conclusions to be drawn from the foregoing experiments as a whole are that, clinically, the Australian disease:

1. Bears no likeness-relation to vaccinia in guinea-pigs, possibly some slight likeness-relation to vaccinia in calves, also to variola in calves when the variola has been passed through a monkey; this relationship would seem to be an intermediate one, between vaccinia on one hand and variola on the other. In monkeys Australian disease is practically undistinguishable from vaccinia or variola.

2. From the monkey experiments, which afford the only evidence of value in this respect, the Australian disease bears a slighter immunity relationship to vaccinia and to variola than either vaccinia or variola bear to each other, and this in spite of the fact that the clinical

relationship of the Australian disease shows certain definite signs of being intermediate between vaccinia on the one hand and variola on the other.

#### SUMMARY.

(1) Inoculation of pathologically active crusts on guinea-pigs caused no reaction, and in this respect a marked difference was demonstrated clinically between the disease of which the crusts were a product, and vaccinia.

(2) On calves the disease tended to show some vaccino-variola relationship, from the appearance of its vesicles, but it appeared to be distinct on the one hand from vaccinia, and from variola on the other.

(3) Calf experiments, two only in number, suggested that vaccinia and variola failed to protect against Australian disease, and that Australian disease failed to protect against vaccinia or variola.

(4) On monkeys the vesicles of Australian disease were undistinguishable in appearance from those of vaccinia or variola, but the evidence for immunity relationship between Australian disease and vaccinia and variola was of the slightest; Australian disease affording no protection against vaccinia or variola, vaccinia possibly protecting against Australian disease in 25 per cent. of the cases, and variola possibly protecting against Australian disease in 40 per cent. of the cases. Vaccinia protected against variola in 100 per cent. of the cases, and variola against vaccinia in 50 per cent. of the cases.

## THE SIGNIFICANCE OF STREPTOCOCCI IN WATER SUPPLIES.

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THE determination of the presence or absence of *B. coli* in a drinking water is universally regarded as the estimation of most value in determining its safety or as grounds for its condemnation. Valuable as this estimation is it is desirable that, if possible, it should be reinforced and confirmed by the determination of the presence or absence of other organisms. In particular it would be of extreme value and importance if the isolation of other selected organisms could be used to throw light upon two points in regard to which evidence is either lacking or indefinite at the present time. These two matters are the question as to whether ascertained contamination is of human or animal origin and as to the probable date of any contamination shown to be present. The *B. coli* enumeration does throw considerable light upon the latter, but none upon the former point.

It is important to consider to what extent the determination of the streptococcus content of a water is of value in the following connections:

- (a) To enable an opinion to be given as to the purity of a given water supply and in confirmation of other bacteriological determinations.
- (b) To supply information as to the actual source of any pollution found to occur, particularly whether human or animal in origin.
- (c) To throw light upon the interval which has elapsed since such bacterial contamination occurred.

A number of investigations have been carried out in connection with these different aspects of the subject. It has been established that streptococci as a class are abundant in sewage and in excreta, both human and animal. Further a number of workers have failed to find these organisms in water supplies known to be pure while they can be



found, often in large numbers, in water supplies known to be definitely sewage contaminated.

A certain amount of investigation has also been undertaken to study the varieties of streptococci found in waters with the object of ascertaining the strains which are specially associated with excretal contamination, particularly those of human origin. It cannot be said that these researches have yielded data which are available to frame an opinion either as to the recentness of any pollution or its specific source. While it is of value for research purposes and with the hope of ultimate success along these lines, to isolate and investigate the different strains of streptococci in water supplies, the determination of the characters of isolated streptococci gives no information additional to that yielded by the numerical estimation of their presence as a class.

While from a broad and general point of view it may be said that the streptococcus determination has been shown to be of value there is as yet by no means a consensus of opinion as to whether this determination is of sufficient value to make it worth while to carry it out. Indeed many bacteriologists omit the examination altogether, while others who carry it out pay but little attention to the findings when obtained. Very few series of analyses have been published in which streptococcus enumerations have been recorded along with topographical details and the *B. coli* and other bacterial determinations.

In our opinion it is desirable to carefully consider the precise value of this estimation and in this paper we record data dealing with a large series of individual samples. The results deal entirely with the presence of streptococci as a class and not any particular varieties. Some questions dealing with the comparative vitality of streptococci and *B. coli* organisms in natural waters are being investigated, and we hope to publish the results shortly.

#### METHOD OF EXAMINATION AND NUMERICAL DETERMINATION.

It is a decided drawback to the streptococcus test that none of the available methods of examination are really satisfactory. Two types of method have been recommended—direct and indirect.

The direct method involves the concentration of the water by filtration through porcelain filters, or by other means, and brushing definite fractions of the concentrated water over suitable solid media in Petri dishes. The streptococci are isolated from the plates and if necessary studied in pure culture. This procedure requires a considerable quantity

of the water, is troublesome and time consuming, and the isolation of the individual streptococcus colonies is unsatisfactory. For quantities of 1 c.c. or less, which can be plated direct, this procedure is useful but in our opinion is very unsatisfactory for larger quantities of water. In the indirect method the water is added in varying amounts to liquid media. After incubation the sediment is examined for streptococcus chains, and if necessary these may be isolated on solid media and their characters studied.

Glucose formate broth incubated anaerobically has been recommended as suitable for this purpose, but we have used glucose neutral red broth incubated aerobically. This method has been used by one of us for many years<sup>1</sup>. The exact procedure used is as follows: 0.1 and 1.0 c.c. respectively of the water are added to tubes of glucose neutral red broth while 10 c.c. is added to a tube of the same broth but of double strength. The larger quantity of the water tested, *i.e.* 30 c.c., is mixed with a large tube of the double strength broth. The broth mixtures are incubated at 37° C. for 40–48 hours and are then examined for streptococci in hanging-drop preparations. Only cocci in quite definite chains are taken as evidence of the presence of streptococci, and if none are found several preparations are examined from each tube before a negative result is recorded.

In the rare cases in which doubt arises as to whether streptococci chains are present it may be necessary to centrifugalise and stain the deposit.

Neutral red is preferred to plain neutral broth since the presence of the dye facilitates the detection of the streptococci in the microscopic preparations.

The method is obviously open to several objections of which the two most important are that delicate strains of streptococcus may not develop in the medium selected and these or even ordinary strains may be overgrown by other bacteria, and that the value of the method is too much dependent upon the care and attention of the individual carrying out the examination. It has the very great merit that it is simple and easy to carry out and takes up very little time or material.

<sup>1</sup> See *Bacteriological Examination of Water Supplies*, by William G. Savage, 1906, Lewis & Co.

RESULTS OBTAINED FROM THE EXAMINATION OF DIFFERENT  
WATER SUPPLIES.

The following results are based upon bacteriological analyses of drinking waters made in the Somerset County Laboratory during the last four to five years and carried out by the method described above, which was identical throughout the period of examination.

While a large proportion of the samples were from sources the sanitary circumstances of which were within our personal cognizance this is not the case for many of them and we are on this account precluded from adopting local topographical features as our main basis of classification. Failing this the most profitable method of comparison and classification is to compare the streptococcus findings with the numerical presence of *B. coli* in the same samples, using the sanitary information available to elucidate particular samples.

The data available can be conveniently dealt with in two groups:

I. Comparison of the results in bulk.

II. Consideration of individual supplies and groups of supplies.

I. *Comparison of the results in bulk.*

In view of the different significance to be attached to the presence of streptococci and *B. coli* in different classes of waters it is necessary to consider these classes separately. So few upland surface water samples were available in the present series of analyses that we have decided not to deal with this group and all such analyses have been excluded. The samples included in the present paper are grouped into the two following classes:

A. *Deep water supplies.* This group includes the samples from springs and deep wells.

In regard to these samples it must be taken into consideration that while they must be classed from the point of view of their origin and main source as not derived from the superficial strata many of them at the time of their examination received, or were liable to receive, an admixture of surface water. Indeed the reason why many of these samples were submitted for examination was because surface contamination was suspected and in many cases the analytical results showed that this in fact did occur.

B. *Surface water supplies.* Practically all these samples were obtained from surface (or shallow) wells, including the ordinary draw wells with open mouth and bucket, surface wells with pumps and dip wells.

Here again this statement must be qualified by stating that while all the samples were classed as of this character and so described by the officials who collected them, it is probable that for a few of them the wells although not sunk to any great depth really penetrated the upper impervious layer and their water was derived from the deeper pervious strata beneath.

For purposes of comparison the results are classified on a *B. coli* basis as follows:

<i>B. coli</i> present in	Group A samples	Group B samples
0.1 or 1 c.c.	Evidence of marked contamination.	Evidence of marked contamination.
10 c.c.	Highly suspicious and showing evidence of contamination.	Of suspicious and doubtful quality.
30 c.c.	Ditto.	Passed as showing no definite evidence of contamination.
Absent in 50 c.c.	No evidence of undesirable bacterial contamination.	No evidence of undesirable bacterial contamination.

The characters relied upon for the determination of *B. coli* were gas production in lactose bile salt broth, the character of the growth upon gelatine slope with absence of liquefaction (2 weeks), the production of indol in peptone water and the production of acid and clot in litmus milk within a week.

The findings in 340 samples of Class A and 974 of Class B, grouped according to their *B. coli* and streptococcus determinations, are shown in Tables I and II.

TABLE I.

A. *Deep water supplies.*

<i>B. coli</i> in	Streptococci present in			Absent from 40 c.c.	Totals
	0.1 or 1	10	30 c.c.		
0.1 or 1 c.c.	22	27	12	6	67
10 or 30 c.c.	3	22	35	58	118
Absent from 50 c.c.	1	4	16	134	155
Totals	26	53	63	198	340

B. *Surface supplies.*

<i>B. coli</i> in	Streptococci present in			Absent from 40 c.c.	Totals
	0.1 or 1	10	30 c.c.		
0.1 or 1 c.c.	234	142	39	28	443
10 c.c.	46	88	60	55	249
30 c.c. or absent from 50 c.c.	9	51	63	159	282
Totals	289	281	162	242	974



TABLE II (*Percentages*).A. *Deep water supplies.*

<i>B. coli</i> in	Streptococci present in				Absent from 40 c.c.
	0.1 or 1	10	30 c.c.		
0.1 or 1 c.c.	84.6 32.8	51 40.3	19 17.9	3 9	
10 or 30 c.c.	11.5 2.5	41.5 18.6	55.5 29.6	29.3 49.2	
Absent from 50 c.c.	3.8 0.6	7.5 2.5	25.4 10.3	67.7 86.5	

B. *Surface supplies.*

<i>B. coli</i> in	Streptococci present in				Absent from 40 c.c.
	0.1 or 1	10	30 c.c.		
0.1 or 1 c.c.	81 52.8	50.5 32	24 8.8	11.6 6.3	
10 c.c.	16 18.5	31.3 35.3	37 21.1	22.7 22.1	
30 c.c. or absent from 50 c.c.	3 3.2	18.2 18.0	39 22.3	65.7 56.4	

In calculating percentages nothing is to be gained by working out the percentage of water samples which contain streptococci or *B. coli* respectively in the different amounts. The percentage results are worked out in two ways. In one way (figures in italic type, Table II) each group of *B. coli* prevalence is taken separately and the percentage prevalence of each group of waters on a streptococcus basis is calculated. In the other way (figures in ordinary type, Table II) each group of streptococcus prevalence is taken separately and the percentage prevalence of the waters on a *B. coli* basis is recorded.

Certain broad deductions may be made from these tables.

*Absence of streptococci.* In two-thirds of the samples from both groups of waters (67.7 and 65.7 per cent. respectively) in which streptococci were absent this absence was associated either with an absence of *B. coli* or their presence in only very small numbers. For the remaining one-third with absence of streptococci *B. coli* were usually present in small numbers only, but this was not invariably the case and this proportion of samples is sufficiently high (3 per cent. in Group A and 11.6 per cent. in Group B) to suggest that by itself absence of streptococci is insufficient to pass a particular sample as satisfactory although a point in favour of its purity.

Consideration of the detailed analyses of the samples with high *B. coli* content but showing no streptococci and a study of the sanitary surroundings (when information was available) showed that in fact all or nearly all these supplies must be considered as contaminated.

It may be that streptococci were really absent from these samples, but it is probable that in some cases, possibly for most of them, the technique was at fault and streptococci present in the water as collected

failed to grow in the culture tubes either from the competition of other organisms or from inherent delicacy.

*Streptococci present in comparatively small numbers.* When found only in 30 c.c. the findings are fairly in accord with those of *B. coli*, but since in the surface supplies (Group B) the presence of *B. coli* in 30 c.c. is grouped with absence of *B. coli* the streptococcus findings are higher in this group than for Group A.

The presence of streptococci in 10 c.c. was in general associated with samples of bad bacterial quality on a *B. coli* basis. Thus 92.5 per cent. of the deep water samples in which streptococci were present in 10 c.c. contained *B. coli*, while 81.8 per cent. of the surface supplies contained *B. coli* in 10 c.c. or less. All these samples on a *B. coli* basis would be regarded as of bad bacteriological quality.

*Streptococci in large numbers (i.e. in 0.1 or 1 c.c.).* These findings are closely in accord with the *B. coli* results. In only 3 and 3.8 per cent. respectively were abundant streptococci met with in samples which were satisfactory on a *B. coli* basis. These percentages represent one actual sample in Group A and nine in Group B. The details of these ten samples are shown in Table III.

The percentage results may also be considered from the *B. coli* point of view.

*Absence of B. coli or presence in only small numbers.* Streptococci were only found in 10 c.c. or less in five (i.e. 3.1 per cent.) of the deep water samples which were free from *B. coli*, a very close agreement. The details of these five samples are shown in Table III.

The agreement is less close for the surface supplies since 21.2 per cent. of the samples in which *B. coli* was either absent or in 30 c.c. only contained streptococci in 10 c.c. or less. Of these in nine samples (shown in Table III) streptococci were in 0.1 or 1.0 c.c. A closer parallel is however found if the 156 samples free from *B. coli* in 50 c.c. are considered separately. Twenty samples (13 per cent.) contained streptococci in 10 c.c. and only four (2.5 per cent.) in 0.1 or 1 c.c. Assuming a value to the streptococcus determinations these results suggest that the permissible standard of *B. coli* in 30 c.c. for this class of waters is probably often a too lenient one.

*Waters decidedly suspicious on a B. coli basis.* In 49.2 per cent. of the deep water samples classed as suspicious on a *B. coli* enumeration, streptococci were not found, furnishing further evidence in favour of the view that absence of streptococci is not in itself a reliable criterion of purity. Of the remaining 50.7 per cent. in 48.2 per cent. the streptococcus

and *B. coli* results were the same while in 2.5 per cent. streptococci were very abundant (in 0.1 or 1 c.c.). Of the surface supplies decidedly suspicious on a *B. coli* basis, streptococci were absent in 22.1 per cent., in 10 or 30 c.c. in 59.4 per cent. and very abundant in 18.5 per cent.

*Samples showing marked evidence of contamination on a B. coli basis.*

The tables show that the majority showed abundant streptococci, but sometimes only in 10 c.c., while in 9 per cent. of the deep water samples and 6.3 per cent. of the surface samples no streptococci were found.

TABLE III.

No.	Source	Organisms per c.c.		Per litre		Remarks
		37° C.	21° C.	<i>B. coli</i>	Streptococci	
1.	Deep well	220	350	Absent*	1000-10,000	See No. 53, Table IV.
2.	Spring	20	800	"	100-1000	Some lactose fermenters in 30 c.c., but no <i>B. coli</i> . A spring rising through boggy land.
3.	"	40	2000	"	"	Liable to surface contamination from a road.
4.	"	15	20	"	"	A public water supply from springs from Old Red Sandstone. Other examinations satisfactory.
5.	"	1140	—	"	"	A spring about 30 yards from house.
6.	Borehole	2500	—	Over 10,000	Absent*	Sample taken soon after the boring made.
7.	Spring	30	200	1000-10,000	"	
8.	Deep well	57	170	"	"	See No. 51, Table IV. Streptococci found in 500 c.c.
9.	Spring	7	10	"	"	Sample taken after passage through old and probably defective pipe.
10.	"	15	30	"	"	See No. 19, Table IV.
11.	"	350	6000	"	"	See No. 29, Table IV.
12.	Shallow well	200	—	Absent	1000-10,000	Atypical organisms, giving no indol and only slightly fermenting lactose. isolated from 30 c.c.
13.	"	56	—	"	"	No true <i>B. coli</i> , but very aberrant lactose fermenters.
14.	"	120	—	"	"	
15.	"	600	—	"	"	Very atypical forms isolated from 1 and 10 c.c. Collected after heavy rain. An open draw well about 20 ft deep in Keuper marl and in unsatisfactory position.
16.	"	240	—	30-100	Over 10,000	A well in a back yard.
17.	"	320	—	"	1000-10,000	
18.	"	600	1200	"	"	
19.	"	145	—	"	"	Well with pump but defective cover. Surrounded by cultivated garden land. Also possible contamination from slop drainage.
20.	"	25	500	"	"	Acid and gas in 10 c.c. bile salt broth, but no <i>B. coli</i> could be isolated.

\* "Absent" means absent from 50 and 40 c.c. respectively.

II. *Consideration of individual supplies and groups of supplies.*

The actual proportion of cases in which the *B. coli* and streptococcus results are widely discrepant has been shown in the tables to be a low one. In Table III the most important of the discrepant individual analyses have been collected together.

Several of the samples in Table III are from supplies which are set out in subsequent tables and are more conveniently dealt with when these are being discussed. It will be noted in a good many of the samples showing abundant streptococci, but no *B. coli* in 50 c.c., that lactose fermenters were present but of types which precluded them being considered as *B. coli* even with a lax interpretation. Information in regard to these supplies was in many cases available and makes it probable that most of them were liable to contamination. For these the streptococcus determinations the more accurately mirror the quality of the supplies.

The significance of streptococci in water can also be studied by considering series of analyses from individual supplies.

A. *Deep water supplies.*

A few supplies have been selected which have been examined on a number of occasions and the local conditions of which are well known to us. The details of the analyses are set out in Table IV.

TABLE IV.

Supply	No.	Date examined	Organisms per c.c.		Per litre		Remarks
			37° C.	21° C.	<i>B. coli</i>	Streptococci	
A	1	Oct. 24th, 1911	100	500	1000-10,000	1000-10,000	
"	2	Nov. 13th, "	120	950	"	"	
"	3	Nov. 29th, "	60	—	30-100	Absent	
"	4	Jan. 16th, 1912	40	100	100-1000	"	
"	5	May 6th, "	7	35	Absent	"	Streptococci in 100 c.c., but <i>B. coli</i> absent in 100 c.c.
"	6	June 4th, "	150	605	Over 10,000	Over 10,000	
"	7	Feb. 5th, 1913	3	20	In 100 c.c. only	Absent	
"	8	Oct. 9th, "	26	53	1000-10,000	100-1000	
B	9	Nov., 1911	—	20	Absent	Absent	
"	10	June, 1912	6	20	30-100	"	
"	11	Dec., "	2	12	Absent	"	
"	12	March, 1914	1	6	"	"	
"	13	Feb., 1915	1	15	"	"	



TABLE IV—(continued).

Supply	No.	Date examined	Organisms per c.c.		Per litre		Remarks
			37° C.	21° C.	<i>B. coli</i>	Streptococci	
C	14	Aug., 1911	40	300	100-1000	Absent	
	15	Nov., "	—	650	1000-10,000	30-100	
	16	Nov., "	18	350	100-1000	100-1000	
	17	April, 1912	22	40	Absent	Absent	
	18	June, "	130	950	Over 10,000	100-1000	
	19	June, 1913	15	30	1000-10,000	Absent	
	20	Sept., "	50	500	1000-10,000	1000-10,000	
	21	June, 1914	7	120	100-1000	30-100	
	22	Sept., "	12	35	"	100-1000	
	23	Dec., "	42	1350	"	"	
D	24	June, 1915	4	42	30-100	Absent	
	25	June, 1912	8	360	100-1000	30-100	
	26	Aug., "	37	350	30-100	100-1000	
	27	Sept., "	12	55	"	30-100	
	28	Nov., "	60	240	Absent	"	
	29	Oct., 1913	350	6000	1000-10,000	Absent	
	30	Dec., 1911	200	750	100-1000	30-100	Not collected so as to avoid mixture with surface water.
E	31	April, 1912	9	95	Absent	Absent	Personally collected.
	32	Dec., 1913	3	32	"	"	
	33	March 1914	2	9	"	"	
	34	July, 1911	10	1200	"	30-100	
F	35	July, "	3	—	100-1000	Absent	<i>B. coli</i> rather atypical.
	36	Oct., 1913	9	46	30-100	"	
	37	Aug. 10th, 1914	11	160	1000-10,000	30-100	
	38	Aug. 26th, "	2	25	30-100	Absent	
	39	Sept., "	95	420	1000-10,000	1000-10,000	
	40	Feb. 11th, 1915	1	2	Absent	Absent	From spring head.
	41	Feb. 11th, "	170	850	100-1000	Over 10,000	After passage through open agricultural pipes for 2 miles.
	42	July, 1912	1	—	Absent	Absent	
G	43	May, 1913	820	2500	"	"	
	44	Aug., 1914	2	4	"	"	
	45	March, 1913	2	14	30-100	"	
H	46	Nov. 3rd, 1914	400	7500	1000-10,000	Over 10,000	
	47	Nov. 9th, "	135	1075	"	100-1000	
	48	Dec. 15th, "	16	312	100-1000	30-100	
J	49	Aug., 1911	20	50	Absent	Absent	
	50	Jan. 1st, 1912	3	2500	100-1000	"	
	51	Jan. 24th, "	57	170	1000-10,000	"	
	52	Feb., "	3	12	Absent	30-100	
	53	March, "	220	350	"	1000-10,000	
	54	June, "	2	25	"	Absent	
	55	Sept., "	2	10	"	"	
	56	Dec., "	5	70	"	"	
	57	April, 1913	15	25	"	"	

TABLE IV—(continued).

Supply	No.	Date examined	Organisms per c.c.		Per litre	
			37° C.	21° C.	<i>B. coli</i>	Streptococci
<i>J</i>	58	Sept., 1913	3	18	Absent	Absent
"	59	Dec., "	2	12	"	"
<i>K</i>	60	Jan. 22nd, 1912	110	250	1000-10,000	30-100
"	61	Feb. 15th, "	40	120	30-100	Absent
"	62	Sept. 9th, 1913	10	250	100-1000	"
"	63	June 10th, 1915	5	40	30-100	"
<i>K</i> <sub>1</sub>	64	Feb. 15th, 1912	4	30	Absent	"
"	65	Dec. 10th, "	72	900	1000-10,000	100-1000
"	66	June 23rd, 1915	3	12	Absent	Absent
<i>K</i> <sub>2</sub>	67	Feb. 15th, 1912	41	120	100-1000	"
"	68	Jan. 2nd, 1913	7	35	30-100	30-100
"	69	Sept. 9th, "	5	105	100-1000	Absent
<i>K</i> <sub>3</sub>	70	Feb. 15th, 1912	9	80	Absent	"
"	71	Jan. 2nd, 1913	18	90	"	"
"	72	June 23rd, 1915	6	26	30-100	"
<i>K</i> <sub>4</sub>	73	Feb. 15th, 1912	3	21	"	"
"	74	June 23rd, 1915	6	35	100-1000	30-100
<i>K</i> <sub>5</sub>	75	Aug. 24th, 1914	135	350	Over 10,000	100-1000
"	76	May 4th, 1915	35	—	100-1000	"
"	77	June 23rd, "	3	16	30-100	Absent

*Addendum to Table IV.*

The following particulars as to these sources of supply are necessary to elucidate the analyses.

*Supply A.* A large and important water supply obtained from a well 36 feet deep with several lateral headings sunk in the Carboniferous Limestone. The supply is liable to considerable intermittent contamination, particularly after heavy rain following a period of dry weather. The limestone is very fissured and the contamination is probably from surface waters through the fissures.

*Supply B.* Obtained from a well and borehole 130 feet deep in the Carboniferous Limestone. The surroundings are quite satisfactory.

*Supply C.* A village supply obtained from a spring. Careful inspections and investigations on the spot, together with the bacteriological and chemical analyses made, show that the spring is liable to contamination with surface water. The water supplied is a mixture of (presumably) pure spring water and surface water more or less bacterially contaminated. The analytical results obtained are largely influenced by the rainfall and when this is heavy, with a consequent heavy admixture of surface water to the spring water, the bacteriological results are bad, usually markedly so.

*Supply D.* From a spring about half-a-mile from the village which it supplies. No local sources of contamination can be found to account for the evidence of pollution nearly uniformly shown by the analyses. We have not however ourselves investigated the surroundings of this supply.

*Supply E.* A large supply obtained from a spring which rises at the junction of the Old Red Sandstone and the Lower Limestone Shales. The surroundings are satisfactory and show no likelihood of contamination.

*Supply F.* This supply is derived from a spring and after Nov., 1912, also from a borehole both in the Dolomitic Conglomerate. The surroundings of the spring and borehole are satisfactory and properly protected, but the water is conveyed by earthenware pipes for a distance of several miles before it is again collected and transmitted to the reservoirs of the town supplied. The majority of the samples are from the water as distributed and after contamination has taken place through the faulty pipes.

*Supply G.* A considerable supply from a well and borehole in the Trias, about 290 feet deep.

*Supply H.* Obtained from a spring from the Dolomitic Conglomerate. The surroundings of the spring are very unsatisfactory and the water is rapidly discoloured after rain.

*Supply J.* Obtained from a well 31 feet deep sunk in the Carboniferous Limestone. At one time the analyses were unsatisfactory as shown in the table and this was traced to contamination from surface waters along the sides of the well. Quite satisfactory analytical results have been obtained after this matter was remedied.

*Supply K.* Water derived from five springs ( $K_1$  to  $K_5$ ) issuing from Devonian rock collected under the soil and passing to a water main which runs along the valley. The pipes are so arranged that the individual springs can be added or cut off as required. Considerable alterations and improvements have taken place during the period covered by the analyses and unsatisfactory agricultural pipes have been replaced by proper iron pipes. In general the surroundings are satisfactory although liable to some contamination from sheep grazing over the gathering area, but the means of collecting the water at the time the early samples were collected were not good. The samples  $K$  are from the mixed waters  $K_1$ ,  $K_2$ , etc. from the individual springs.

Many of these supplies have been selected for this table because they have been from time to time, or continuously, contaminated by surface water or from other undesirable sources and it is of interest to note how far such contamination is shown by both the *B. coli* and streptococcus enumerations.

Table IV shows that with a few exceptions the correspondence is a close one. For supplies A and B the agreement is very close. Most of the samples for supply C agree, but on several occasions streptococci were not detected when *B. coli* were abundant. None of the samples showed abundant streptococci with absent or relatively few *B. coli*.

Supplies E, G and H agree closely as regards these two determinations, but for D and F a few of the samples showed considerable differences. Supply J is of great interest since in the early part of the year 1912 the *B. coli* findings (but not the streptococcus estimations) showed marked contamination. This was traced to contamination from surface water

along the sides of the well. Steps were taken to prevent this and later analyses were quite satisfactory. Sample No. 53 was taken very soon after the alterations had been made and although *B. coli* were absent streptococci were abundant. The high 21° or 37° counts confirm the streptococcus findings and probably here the streptococcus count was more reliable than the absence of the *B. coli* and was of decided utility.

The results for supplies K mostly agree but as in other cases in several instances absence of streptococci was associated with presence of *B. coli* in small and in three cases in considerable numbers.

### B. Surface water supplies.

Almost all the samples were from shallow wells mostly with a pump, but many were open draw wells, while a few were dip wells.

As a rule each well was examined not more than once, or at the most twice, so that it is not possible to submit series of analyses from individual supplies. Very variable results are liable to be obtained from surface water samples taken on different occasions since the local conditions vary from time to time while, in particular, much depends upon the extent of the previous rainfall.

Table V is of interest from the point of view of comparing the *B. coli* and streptococcus results of surface wells, all from the same parish, re-examined after a long interval. Between the examinations some steps had been taken to improve the condition of the wells, while all the later samples (examined May, 1915) were collected after a long period of dry weather.

TABLE V.

No.	Date examined	No. of organisms at 37° C.	Per litre		Remarks
			<i>B. coli</i>	Streptococci	
1.	July, 1914	10	1000-10,000	Absent	A well about 8 ft deep with pump; position unsatisfactory.
1 a.	May, 1915	3	Absent	"	
2.	August, 1914	25	100-1000	100-1000	Well with pump. Leaky w.c. and drains near well at time of first examination. Position also unsatisfactory in other ways. Drainage put right between the examinations.
2 a.	May, 1915	4	Absent	Absent	
3.	August, 1913	120	"	1000-10,000	Open draw well with defective walls. Position rather unsatisfactory.
3 a.	May, 1915	24	"	Absent	
4.	March, 1914	170	1000-10,000	100-100	Well with pump.
4 a.	May, 1915	12	30-100	Absent	
5.	August, 1913	42	100-1000	30-100	Well with pump. Pump in scullery of the dwelling house. Well not opened, but position and surroundings unsatisfactory.
5 a.	May, 1915	25	1000-10,000	100-1000	
6.	May, 1913	105	"	"	Open draw well about 60 ft deep. 6 ft from house and 60 from privy. Liable to surface contamination.
6 a.	May, 1915	20	Absent	"	



Table VI is given as a good example of a series of samples from surface wells which were all in the same parish and sunk in the same geological strata. Forty-seven different wells were bacteriologically examined, two samples being from the same well. The parish has a population of 550 and is situated in central Somerset on a slight elevation of Keuper marl, the low-lying land round being covered with alluvium and recent deposits. When the samples were collected the parish depended entirely upon surface wells for its water supply. The wells were sunk in the marl, usually to a depth of 20 to 30 ft, and yielded but limited supplies of water. Many of them were inspected by one of us. All these were of the usual type with sides of loose stone not made impervious in any way and not set in cement. Some were open draw wells but most had pumps, many of the latter with faulty coverings to the wells. The majority were in close proximity to the houses they supplied and a good many were in yards or outhouses, or in some cases under the floors of the kitchens or wash-houses. On inspection many could be condemned outright, a few were in reasonably satisfactory positions while the remainder were in positions which rendered the pollution of their contents likely to result but for which bacteriological examinations were necessary to say if it occurred.

In general the streptococcus and *B. coli* results agree closely. Samples Nos. 8 and 9 were markedly contaminated on a *B. coli* basis, but no streptococci were found. We have not examined well No. 8, but the well from which Sample No. 9 was obtained was in a shed, with a proper cement floor, by the side of the house. The well was covered over and provided with a pump and there were no drains near or other obvious sources of contamination apart from the close proximity to the house.

In five samples, *i.e.* Nos. 14, 15, 16, 17 and 18, streptococci were present only in small numbers while *B. coli* were very abundant. Sample No. 14 is from the same well as Sample No. 27, in which fairly numerous streptococci were found. Sample No. 16 was obtained from a well with a pump, but the position was one liable to marked pollution and the well was condemned on this ground alone. No. 17 was from a well with a pump by the side of the house. The ground round was dirty and there was considerable probability of contamination. No. 18 was a draw well showing no direct evidence of sources of contamination apart from the open mouth. Samples Nos. 18 and 19 were from wells which inspection showed were unsatisfactory as regards their position and surroundings and the streptococcus results are probably more reliable than the *B. coli* findings. In No. 20 lactose fermenters were present

*Streptococci in Water Supplies*

TABLE VI.

No.	Organisms per c.c. at 37° C.	Per litre	
		<i>B. coli</i>	Streptococci
1.	55	Absent	Absent
2.	40	"	"
3.	65	"	"
4.	8	30-100*	"
5.	18	"	"
6.	70	"	"
7.	23	"	"
8.	450	1000-10,000	"
9.	260	"	"
10.	20	100-1000*	30-100
11.	1800	"	"
12.	95	"	"
13.	650	"	"
14.	220	1000-10,000	"
15.	12	"	"
16.	450	" *	"
17.	400	Over 10,000	"
18.	1120	" *	"
19.	220	Absent	100-1000
20.	180	"	"
21.	140	30-100	"
22.	710	"	"
23.	200	100-1000*	"
24.	320	"	"
25.	320	"	"
26.	125	" *	"
27.	85	"	"
28.	7	1000-10,000	"
29.	640	"	"
30.	300	" *	"
31.	45	"	"
32.	700	"	"
33.	1200	"	"
34.	1200	"	"
35.	650	" *	"
36.	260	"	"
37.	1700	Over 10,000	"
38.	90	"	"
39.	700	"	"
40.	200	1000-10,000	1000-10,000
41.	720	" *	"
42.	880	"	"
43.	270	"	"
44.	450	Over 10,000	"
45.	4000	"	"
46.	520	"	Over 10,000
47.	50,000	"	"
48.	520	"	"

\* The isolated *B. coli* were indol negative, otherwise typical.

in 10 and 1 c.c., but they were not classed as *B. coli*, failing to clot milk or produce indol, while the growth on gelatine slope was of unusual type. Probably a further sample would have shown numerous typical *B. coli*.

The majority of the samples in Table VI show evidence of contamination. In contrast to this the second series of wells shown in Table VII may be considered. The samples were all collected from the wells in

TABLE VII.

No.	Organisms per c.c. at 37° C.	Per litre	
		<i>B. coli</i>	Streptococci
1.	5	Absent*	Absent*
2.	5	"	"
3.	2	"	"
4.	8	"	"
5.	3	"	"
6.	22	"	"
7.	2	"	"
8.	8	"	"
9.	7	"	"
10.	6	"	"
11.	12	"	"
12.	8	Absent (atypical 30-100)	"
13.	5	Absent (atypical 30-100)	"
14.	7	30-100	"
15.	110	100-1000†	"
16.	60	"	"
17.	2500	Over 10,000	"
18.	7	Absent	30-100
19.	15	"	"
20.	8	"	"
21.	88	100-1000	"
22.	16	100-1000†	"
23.	15	1000-10,000	"
24.	200	Absent	100-1000
25.	500	30-100	"
26.	15	"	"
27.	55	"	"
28.	50	100-1000	"
29.	35	"	"
30.	35	1000-10,000	"
31.	95	"	"
32.	300	Over 10,000	"
33.	150	100-1000	1000-10,000
34.	800	1000-10,000	"
35.	640	Over 10,000	"
36.	520	"	"

\* "Absent" = absent from 50 or 40 c.c. respectively.

† *B. coli* indol negative.

a small country town of about 4600 population, provided with a pure water supply from springs but which also contained a considerable number of surface wells which the occupiers preferred to use to the town supply. The essential object for the collection of the samples was to ascertain if these wells should be closed. Almost all had pumps provided.

In general the *B. coli* and streptococcus results show a large measure of agreement the greatest differences being met with in Samples Nos. 15, 16, 17, 23, 24 and 25.

A third series of analyses of the water of surface wells from another parish is shown in Table VIII. They are reproduced here as the sanitary surroundings of every well were carefully investigated by us on more than one occasion. The parish is a somewhat scattered one and the wells are at very different levels. Some are 50 or more feet deep, the level of the subsoil water being at least 30 ft from the surface when the samples were taken, while others are in low-lying parts, the water level being but a foot or so from the ground level and sunk in the alluvium. In winter the latter are usually flooded.

All the wells were of defective construction and all in such unsatisfactory surroundings that sanitary inspection alone was sufficient to condemn them.

TABLE VIII.

No.	Organisms per c.c. at 37° C.	Per litre		Remarks
		<i>B. coli</i>	Streptococci	
1.	50	1000-10,000	Absent	<i>B. coli</i> indol negative. A rather deep well with pump.
2.	80	30-100	30-100	Pump.
3.	50	100-1000	"	"
4.	10	1000-10,000	"	Draw well.
5.	400	Absent	100-1000	"
6.	50	30-100	"	A dip well fed by a superficial spring but liable to gross surface contamination.
7.	320	100-1000	"	Pump.
8.	200	"	"	Draw well.
9.	550	"	"	"
10.	140	1000-10,000	"	"
11.	30	100-1000	1000-10,000	"
12.	1200	"	"	"
13.	320	"	"	Pump.
14.	330	1000-10,000	"	"
15.	120	Over 10,000	"	"
16.	480	1000-10,000	Over 10,000	Draw well.
17.	5000	Over 10,000	"	"
18.	350	"	"	Pump.
19.	150	"	"	"



It is of interest to note that the streptococcus as well as the *B. coli* findings in nearly every case confirm the results obtained from the inspection of the wells and their surroundings. Only in No. 1 were streptococci not found. This sample was from a well in the higher parts of the parish and on inspection considerably less liable to contamination than most of the others. The *B. coli* content was higher than would have been expected from the inspection. In No. 5 no *B. coli* were found, but some very slow lactose fermenters were isolated. Probably a further sample would have shown *B. coli*. An insanitary privy was near this open draw well and the well was situated immediately at the back of the house. On sanitary grounds the well would be condemned at once. This opinion is confirmed by the high 37° C. count and the abundant streptococci.

The results obtained from the examination of the wells of a number of other parishes have been tabulated and critically examined, but as the evidence they furnish is all on the lines of the above it is unnecessary to reproduce them and multiply the number of tables.

#### CONCLUSIONS.

The results of the analyses studied in bulk and detailed consideration of individual supplies yield results which are essentially the same.

The absence of streptococci (at least with the methods used) is of less significance than their presence. Absence of streptococci, even from a considerable bulk of the water, cannot be accepted, to the same extent as absence of *B. coli*, as reliable evidence of the freedom from serious contamination of the water at the time the particular sample was collected, although it is a point in favour of its purity.

Standards for permissible number of streptococci broadly correspond to similar numerical standards for *B. coli*, but are of less significance and reliability.

While we do not know enough about the varying vitality and distribution of streptococci to say whether the presence of certain strains may or may not be disregarded as evidence of excretal contamination it is, in general, reliable to assume that streptococci in large numbers are only present in waters from unsatisfactory sources.

We are decidedly of opinion that the streptococcus estimation, carried out by the simple method described, is of undoubted value as evidence for or against excretal contamination.

We think further that if the reliability of the method could be improved the value of the streptococcus enumeration would be enhanced and even more clearly demonstrable.

## A CONTRIBUTION TO THE STUDY OF AMBOCEPTORS AND RECEPTORS.

### SECOND COMMUNICATION ON HETEROLOGOUS IMMUNITY TO MALIGNANT MOUSE TUMOURS.

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SINCE Forssman (1911) discovered a heterologous haemolysin obtained by immunising rabbit against guinea-pig organs—kidney, liver, etc.—and having the property of dissolving sheep blood corpuscles but not those of the guinea-pig, many authors have devoted their time to this subject, and to the elucidation of the relationship between antigen and antibody.

Thus, Orudschiew (1913) succeeded in obtaining haemolytic amboceptors by immunising with guinea-pig serum, while Spaet (1914) claimed that the leucocytes of this animal can also evoke the production of the same amboceptors. In 1913 Morgenroth demonstrated two haemolysins obtained by immunising rabbit against mouse kidney and against mouse carcinoma, and noted that it was a difficult task to decide whether the haemolysins were identical or partially different. In a later paper, Morgenroth and Bieling (1915) published a detailed account dealing with this subject. For this purpose they employed the methods of fixation and transgression, and declared that the haemolysins were not identical, but differed from one another to a certain extent.

In a previous paper (1915) I investigated the serological reactions of malignant mouse and rat tumour antisera, *i.e.* haemolysis, haemagglutination, complement fixation, precipitin reaction and cytolysis. My experiments were all made in cases of heterologous immunity and working along bacteriological lines, since in the case of homologous immunity to mouse tumours (*e.g.* immunising mouse against mouse

tumours and certain normal tissues—for example mouse embryo-skin) no specific antibody has yet been found.

In the present paper the investigations concern the relationship between mouse tumour and guinea-pig kidney haemolysins and goat-erythrocyte amboceptors respectively, according to the methods of Morgenroth and Bieling.

### I. IMMUNISING METHODS.

To immunise with organic cells and obtain heterologous haemolysin many authors have applied the methods, in some cases modified, already used by Forssman. The methods used by Morgenroth and Bieling are very well adapted for the obtaining of exact results. There are, however, in spite of everything, some unavoidable difficulties when immunising with organic and tumour cells, because, apart from the difficulty that exists in obtaining the antigen absolutely free from blood, it is scarcely possible to separate the connective tissue from the parenchyma; an example of this is seen in Bail and Margulies's (1913) use of guinea-pig kidney as antigen.

However, one can probably neglect the influence of connective tissue, because in mouse tumour and guinea-pig kidney the parenchyma is so largely in excess.

Morgenroth and Bieling's methods were therefore adopted and are as follows:

The immunising materials—mouse tumour and guinea-pig kidney—are removed from animals which have been bled out and are cut up under sterile conditions and then washed with normal saline in order to get rid of as much blood as possible. These materials are ground down in a sterile mortar without any addition of sea sand. To the pulp a certain quantity of saline is added and the whole filtered through an ordinary handkerchief under slight pressure. The emulsions thus prepared were injected intraperitoneally into rabbits, twice or three times with intervals of from eight to ten days.

As to the dosage, Forssman had already suggested that a large quantity does not give a proportionately high immunisation, and Morgenroth and Bieling confirming this assertion, used for each injection emulsion corresponding to 1–2 grms. of original material.

Eight days after the last inoculation, the animals were bled from the carotid under narcosis. The immunised sera obtained were inactivated the next day at 56° C. for half-an-hour. To test the

haemolytic reaction 0.5 c.c. of a 5% washed goat erythrocyte suspension was employed. The complement was 1 c.c. of a 1 in 10 dilution of fresh guinea-pig serum, the absolute quantity of complement in each tube thus being 0.1 c.c. The minimal dose which still can cause complete haemolysis under these conditions was named the amboceptor unit.

The degrees of haemolysis were distinguished as follows:

c. = complete	sl. = slight
n.c. = nearly complete	tr. = trace
m. = marked	n. = none

The following protocols show the haemolysin antigenic power of guinea-pig kidney and mouse tumour.

*Immunisation with guinea-pig kidney.*

*Rabbit 1.*

1915.	8th Nov.	5.0 c.c. of 20 % guinea-pig kidney emulsion intraperitoneally.
	15th "	5.0 " " " "
	22nd "	10.0 " " " "
	30th "	Bled out.
	1st Dec.	Amboceptor unit = 0.005.

*Rabbit 2.*

1915.	8th Nov.	5.0 c.c. of 20 % guinea-pig kidney emulsion intraperitoneally.
	15th "	5.0 " " " "
	22nd "	10.0 " " " "
	30th "	Bled out.
	1st Dec.	Amboceptor unit = 0.002.

*Rabbit 3.*

1915.	8th Nov.	5.0 c.c. of 20 % guinea-pig kidney emulsion intraperitoneally.
	15th "	5.0 " " " "
	22nd "	10.0 " " " "
	30th "	Bled out.
	1st Dec.	Amboceptor unit = 0.001.

*Immunisation with mouse tumour.*

For this purpose mouse carcinoma 199 and sarcoma 37 p. which were supplied by the kindness of Dr Murray, the Director of the Imperial Cancer Research Fund, London, were used. The virulence of these tumours is so great that their takes by transplantation are practically 100 per cent., and their proliferative activity so rapid that they reach from 1 grm. to 1.5 grms. two weeks after an inoculation of 0.03 c.c.



*Immunisation with mouse carcinoma 199.**Rabbit 4.*

1915.	19th Nov.	7.0 c.c. of 25 % mouse carcinoma 199 emulsion intraperitoneally.
	30th "	6.0 " " " "
	10th Dec.	10.0 " " " "
	18th "	Bled out.
	19th "	Amboceptor unit = 0.005 c.c.

*Rabbit 5.*

1915.	19th Nov.	5.0 c.c. of 25 % mouse carcinoma 199 emulsion intraperitoneally.
	30th "	6.0 " " " "
	10th Dec.	10.0 " " " "
	18th "	Bled out.
	19th "	Amboceptor unit = 0.006 c.c.

*Rabbit 6.*

1915.	19th Nov.	5.0 c.c. of 25 % mouse carcinoma 199 emulsion intraperitoneally.
	30th "	6.0 " " " "
	10th Dec.	10.0 " " " "
	15th "	Died.

*Immunisation with mouse sarcoma 37 p.**Rabbit 7.*

1915.	2nd Dec.	10.0 c.c. of 25 % mouse sarcoma 37 p. emulsion intraperitoneally.
	10th "	7.0 " " " "
	20th "	10.0 " " " "
	28th "	Bled out.
	29th "	Amboceptor unit = 0.02 c.c.

*Rabbit 8.*

1915.	2nd Dec.	10.0 c.c. of 25 % mouse sarcoma 37 p. emulsion intraperitoneally.
	10th "	8.0 " " " "
	18th "	Bled out.
	19th "	Amboceptor unit = 0.025 c.c.

According to Morgenroth and Bieling, mouse tumour—carcinoma—contains more antigenic receptors than mouse kidney, but from the above experiments, although few in number, the antigenic power of mouse tumour, either carcinoma or sarcoma, does not appear to exceed that of guinea-pig kidney.

## II. FIXATION EXPERIMENTS.

The guinea-pig kidney and mouse tumour emulsions employed in the following experiments were 10 per cent. by weight, and prepared according to Morgenroth and Bieling; that is to say, the materials were removed from animals, which had been bled out, were cut up, washed with normal saline in order to free them from blood as much as possible, and then centrifuged. Thus the organ and tumour cells were separated from the blood corpuscles, 10 per cent. suspensions of the precipitated cells being made by weight with normal saline. I found 2.0 c.c. of these suspensions an adequate amount for fixation with the serum used.

1. Fixation experiments with mouse carcinoma 199 serum. Rabbit No. 4. A.U. = 0.005 c.c.

For control was used, as in Morgenroth and Bieling's experiments, rabbit kidney emulsion prepared in the way already described.

To 1.0 c.c. (200 A.U.) of the inactivated carcinoma serum was added 2.0 c.c. of 10 % carcinoma, guinea-pig and rabbit kidney emulsions respectively, thus making 3.0 c.c. in all:

1.0 c.c. carcinoma serum (200 A.U.) + 2.0 c.c. of 10 % carcinoma emulsion.  
 1.0 „ „ „ „ + 2.0 „ of 10 % guinea-pig kidney emulsion.  
 1.0 „ „ „ „ + 2.0 „ of 10 % rabbit kidney emulsion.

The mixtures were placed for one hour at 37° C. and afterwards centrifuged. The slightly opaque supernatant fluids were then tested haemolytically, using 0.1 c.c. guinea-pig complement and 0.5 c.c. of 5 % goat erythrocyte suspension. After placing for one hour and a half at 37° C., the tubes were kept in the cold room and the result observed the following morning.

The following table shows the results:

TABLE I.

*Mouse carcinoma serum.*

	Absorbed serum used in haemolytic test	Haemolytic strength before absorption	Haemolysis after absorption with		
			Mouse carcinoma	Guinea-pig kidney	Rabbit kidney
1.	1.2 c.c.	80 A.U.	c.	c.	c.
2.	0.6 „	40 „	n.c.	„	„
3.	0.3 „	20 „	n.c.—m.	n.c.	„
4.	0.15 „	10 „	m.	m.	„
5.	0.075 „	5 „	sl.	sl.	„
6.	0.03 „	2 „	sl.—tr.	tr.	„
7.	0.015 „	1 „	tr.—n.	tr.—n.	n.c.
8.	0.01 „	$\frac{2}{3}$ „	n	n.	m

Mouse carcinoma is thus seen to fix very strongly, while guinea-pig kidney was rather feeble, and by calculating according to Morgenroth and Bieling's method, it is found that mouse carcinoma fixed from at least  $\frac{39}{40}$  to  $\frac{79}{80}$  of the amboceptors, namely, from 195 to 197 A.U., while guinea-pig kidney deviated  $\frac{19}{20}$  to  $\frac{39}{40}$ ; namely, from 190 to 195 A.U. The attempt to fix with rabbit kidney, as Morgenroth has already noted, does not succeed, so that 1 amboceptor unit after absorption still caused practically complete haemolysis. Thus it is clear that rabbit kidney does not fix haemolytic amboceptors, in the same way as, according to Forssman, it does not elicit the antibodies.

2. Fixation experiments with guinea-pig kidney serum. Rabbit No. 3. A.U. = 0.001 c.c.

To tubes containing 0.2 c.c. (200 A.U.) of the serum and 0.8 c.c. saline were added 2.0 c.c. of guinea-pig, mouse carcinoma and rabbit kidney emulsions respectively, the total amount thus being 3 c.c.

0.2 c.c. guinea-pig kidney serum (200 A.U.) + 0.8 c.c. saline + 2.0 c.c. guinea-pig kidney emulsion.

0.2 c.c. guinea-pig kidney serum (200 A.U.) + 0.8 c.c. saline + 2.0 c.c. mouse carcinoma emulsion.

0.2 c.c. guinea-pig kidney serum (200 A.U.) + 0.8 c.c. saline + 2.0 c.c. rabbit kidney emulsion.

After treating the mixtures as before, the supernatant fluids were obtained and tested for haemolytic reaction.

TABLE II.  
*Guinea-pig kidney serum.*

	Absorbed serum used in haemolytic test	Haemolytic strength before absorption	Haemolysis after absorption with		
			Guinea-pig kidney	Mouse carcinoma	Rabbit kidney
1.	1.2 c.c.	80 A.U.	c.	c.	c.
2.	0.6 "	40 "	n.c.	"	"
3.	0.3 "	20 "	m.	"	"
4.	0.15 "	10 "	sl.	"	"
5.	0.075 "	5 "	tr.	n.c.	"
6.	0.03 "	2 "	n.	m.	"
7.	0.015 "	1 "	"	tr.	n.c.
8.	0.01 "	$\frac{2}{3}$ "	"	n.	m.

The fixing power of the guinea-pig kidney emulsion for the amboceptors of the homologous serum lies between  $\frac{79}{80}$  and  $\frac{39}{40}$ , whilst that of the carcinoma emulsion lies between  $\frac{9}{10}$  and  $\frac{4}{5}$ ; in other words, although the carcinoma antigens were capable of fixing haemolytic antibodies, these were most strongly fixed by homologous antigens. Rabbit kidney again showed no power to absorb the haemolysin, so

that one amboceptor unit, after treatment, still caused nearly complete haemolysis. However, the behaviour of goat haemolysin, with mouse carcinoma and guinea-pig kidney emulsion, is quite different.

3. Fixation experiments with goat haemolysin. A.U. = 0.0005 c.c.

The mixture this time consisted of 0.1 c.c. (200 A.U.) of the goat haemolysin, 0.9 c.c. saline and 2.0 c.c. of goat erythrocytes (10 % by weight), guinea-pig and rabbit kidney suspensions respectively. After treating the mixtures as before, the haemolytic power of the supernatant fluids was tested.

TABLE III.

*Goat red cell serum.*

	Absorbed serum used in haemolytic test		Haemolytic strength before absorption	Haemolysis after absorption with			
				Goat erythrocytes	Mouse carcinoma	Guinea-pig kidney	Rabbit kidney
1.	0.6	c.c.	40 A.U.	c.	c.	c.	c.
2.	0.3	"	20 "	"	"	"	"
3.	0.15	"	10 "	m.	"	"	"
4.	0.075	"	5 "	sl.	"	"	"
5.	0.03	"	2 "	n.	"	"	"
6.	0.015	"	1 "	"	n.c.	"	"
7.	0.0075	"	$\frac{1}{2}$ "	"	m.	m.	n.c.
8.	0.0004	"	$\frac{1}{4}$ "	"	tr.	tr.	sl.

As the table demonstrates, goat blood cells fix the amboceptors well, while not only rabbit kidney, but carcinoma and guinea-pig kidney do not absorb them practically at all. This result is very curious, because both mouse carcinoma and guinea-pig kidney contain antigen for the production of amboceptors haemolytic to goat red cells. To explain this fact, the following statement is probably admissible: goat erythrocytes contain many groups of receptors able to produce amboceptors haemolytic to goat blood cells, while mouse carcinoma and guinea-pig kidney contain some of these groups only.

If, therefore, haemolytic serum produced by immunisation with goat blood cells is absorbed with mouse carcinoma or guinea-pig kidney emulsions, those amboceptor groups corresponding to their own receptors are fixed, while there still remain the other groups of antibodies to cause haemolysis. It is certainly due to a want of any receptors in common that rabbit kidney did not fix goat haemolysin amboceptors.

Another question approached in this paper is concerned with the fixing power of goat blood corpuscles for the haemolytic amboceptors of mouse carcinoma and guinea-pig kidney serum. Although Morgenroth and Bieling observed that goat erythrocytes fix the haemolytic



antibodies of mouse carcinoma serum more strongly than mouse kidney emulsion, they did not publish an exact account.

#### 4. Fixation experiments with goat blood cells.

To 1.0 c.c. (200 A.U.) of mouse carcinoma serum and 0.2 c.c. (200 A.U.) of guinea-pig kidney, plus 0.8 c.c. saline, 2.0 c.c. of 10 % goat erythrocytes were added, while the same amount of mouse carcinoma and guinea-pig kidney emulsion was added, each to its corresponding serum as control.

1.0 c.c. mouse carcinoma serum (200 A.U.) + 2.0 c.c. of 10 % goat blood cells.

1.0 „ „ „ „ „ + 2.0 „ of 10 % mouse carcinoma emulsion.

0.2 „ guinea-pig kidney serum (200 A.U.) + 0.8 c.c. saline + 2.0 c.c. of 10 % goat blood cells.

0.2 c.c. guinea-pig kidney serum (200 A.U.) + 0.8 c.c. saline + 2.0 c.c. of 10 % guinea-pig kidney emulsion.

The mixtures were treated as before and the haemolytic reaction of the supernatant fluids tested.

TABLE IV. A.

#### *Mouse carcinoma serum.*

	Absorbed serum used in haemolytic test	Haemolytic strength before absorption	Haemolysis after absorption with	
			Goat erythrocytes	Mouse carcinoma (control)
1.	1.2 c.c.	80 A.U.	m.	c.
2.	0.6 „	40 „	sl.	n.c.
3.	0.3 „	20 „	tr.	n.c.—m.
4.	0.15 „	10 „	„	m.
5.	0.075 „	5 „	n.	sl.
6.	0.03 „	2 „	„	sl.—tr.
7.	0.015 „	1 „	„	tr.—n.
8.	0.01 „	$\frac{2}{3}$ „	„	n.

TABLE IV. B.

#### *Guinea-pig kidney serum.*

	Absorbed serum used in haemolytic test	Haemolytic strength before absorption	Haemolysis after absorption with	
			Goat erythrocytes	Guinea-pig kidney (control)
1.	1.2 c.c.	80 A.U.	n.c.	c.
2.	0.6 „	40 „	m.	n.c.
3.	0.3 „	20 „	sl.	m.
4.	0.15 „	10 „	tr.	sl.
5.	0.075 „	5 „	n.	tr.
6.	0.03 „	2 „	„	n.
7.	0.015 „	1 „	„	„
8.	0.01 „	$\frac{2}{3}$ „	„	„

As the preceding experiments demonstrate, goat blood cells have a much greater fixing power for both haemolytic amboceptors than their homologous antigens, this being particularly the case with the carcinoma amboceptors.

It has been already stated that rabbit kidney fixes the amboceptors of neither mouse carcinoma, guinea-pig kidney, nor goat erythrocyte antiserum, and the immunising of rabbit with rabbit kidney cells conversely does not elicit the production of haemolysins. Consequently, it is natural to suppose that the organs of other animals, for example rat kidney which, according to Forssman and Morgenroth, does not contain the haemolysin antigen, cannot have any fixing power for the amboceptors of mouse carcinoma, guinea-pig kidney and goat erythrocyte antisera.

#### 5. Fixation experiment with rat kidney emulsion.

The experiment was performed as follows:

- A. 0.5 c.c. (100 A.U.) mouse carcinoma serum + 0.5 c.c. saline + 2.0 c.c. rat kidney emulsion.
- B. 0.1 c.c. (100 A.U.) guinea-pig kidney serum + 0.9 c.c. saline + 2.0 c.c. rat kidney emulsion.
- C. 0.1 c.c. (200 A.U.) goat haemolysin + 0.9 c.c. saline + 2.0 c.c. rat kidney emulsion.

These mixtures were treated as before and the haemolytic reaction of the supernatant fluids tested.

TABLE V. A.

#### *Mouse carcinoma serum.*

	Absorbed serum used in haemolytic test	Haemolytic strength before absorption	Haemolysis after absorption with	
			Mouse carcinoma	Rat kidney
1.	1.2 c.c.	40 A.U.	c.	c.
2.	0.6 "	20 "	n.c.	"
3.	0.3 "	10 "	"	"
4.	0.15 "	5 "	m.	"
5.	0.075 "	2½ "	sl.	"
6.	0.03 "	1 "	sl.—tr.	"
7.	0.015 "	½ "	n.	n.c.
8.	0.01 "	⅓ "	"	sl.

TABLE V. B.

#### *Guinea-pig kidney serum.*

	Absorbed serum used in haemolytic test	Haemolytic strength before absorption	Haemolysis after absorption with	
			Guinea-pig kidney	Rat kidney
1.	1.2 c.c.	40 A.U.	c.	c.
2.	0.6 "	20 "	n.c.	"
3.	0.3 "	10 "	m.	"
4.	0.15 "	5 "	sl.	"
5.	0.075 "	2½ "	tr.	"
6.	0.03 "	1 "	n.	"
7.	0.015 "	½ "	"	n.c.
8.	0.01 "	⅓ "	"	m.

TABLE V. C.  
*Goat erythrocyte serum.*

	Absorbed serum used in haemolytic test	Haemolytic strength before absorption	Haemolysis after absorption with	
			Goat erythrocytes	Rat kidney
1.	0.6 "	40 A.U.	c.	c.
2.	0.3 "	20 "	"	"
3.	0.15 "	10 "	m.	"
4.	0.075 "	5 "	sl.	"
5.	0.03 "	2 "	n.	"
6.	0.015 "	1 "	"	"
7.	0.0075 "	$\frac{1}{2}$ "	"	m.
8.	0.004 "	$\frac{1}{4}$ "	"	sl.

The tables show that the supposition was right, that is to say, rat kidney emulsion does not fix the amboceptors practically at all; so that one amboceptor unit in all cases still caused after treatment nearly complete haemolysis. So, it is confirmed that, as Morgenroth asserted, the fixation reaction is specific.

The mouse tumour used in the foregoing experiments has throughout been carcinoma.

It still remained to be seen whether mouse sarcoma serum would behave in the same way. For this purpose serum 37 p. was used, and exactly the same method applied as before.

6. Fixation experiment. Amboceptor unit of serum 37 p.=0.02 c.c.

It is regrettable that this time I could not for certain reasons use homologous tumour emulsion; mouse carcinoma emulsion had to be employed instead.

A. 2.0 c.c. (100 A.U.) of the serum + 2.0 c.c. mouse carcinoma emulsion.

B. 2.0 c.c. (100 A.U.) of the serum + 2.0 c.c. guinea-pig kidney emulsion.

Here the mixtures amounted to 4.0 c.c., it being necessary to take 2.0 c.c. of the serum in order to obtain 100 amboceptor units.

After absorption the haemolytic reaction was carried out on the supernatant fluids.

TABLE VI.  
*Mouse sarcoma serum.*

	Absorbed serum used in haemolytic test	Haemolytic strength before absorption	Haemolysis after absorption with	
			Mouse carcinoma	Guinea-pig kidney
1.	0.8 c.c.	20 A.U.	c.	m.
2.	0.4 "	10 "	n.c.	"
3.	0.2 "	5 "	m.	sl.
4.	0.1 "	$2\frac{1}{2}$ "	m.—sl.	tr.
5.	0.04 "	1 "	sl.	n.
6.	0.02 "	$\frac{1}{2}$ "	tr.	"

It is seen that guinea-pig kidney fixes the amboceptors of mouse sarcoma serum better than mouse carcinoma. In other words, it seems that the former contains more lysinogen than the latter for the amboceptors of sarcoma 37 p. antiserum.

### III. TRANSGRESSION EXPERIMENTS.

The cell-sediments produced by centrifugation (either mouse tumour or guinea-pig kidney) were carefully separated from their supernatant fluids and 10 % suspensions prepared each time before the experiment. The only difference was that the quantity of suspension consisted of 2.0 c.c. while Morgenroth and Bieling used 2.5 c.c.

#### 1. Transgression experiment with mouse carcinoma serum.

Here the sediment obtained in fixation experiment 1, whose protocol is produced here, was used.

1.0 c.c. (200 A.U.) mouse carcinoma serum + 2.0 c.c. carcinoma 199 emulsion = 3.0 c.c.

1.0 c.c. (200 A.U.) mouse carcinoma serum + 2.0 c.c. guinea-pig kidney emulsion = 3.0 c.c.

To the sediments produced by centrifugation was added saline to the original volume (3.0 c.c.). With diminishing amounts of these cell suspensions 0.5 c.c. of 5 % goat erythrocytes was mixed; after incubating at 37° C. for one hour, 0.1 c.c. complement was added, and the tubes again placed in the hot room for two hours.

The following table shows the results:

TABLE VII.

	Amount of cell suspension	Haemolysis after transgression with	
		Mouse carcinoma	Guinea pig kidney
1.	1.0 c.c.	sl.—n.	n.c.
2.	0.5 „	tr.	m.
3.	0.3 „	n.	„
4.	0.2 „	„	sl.
5.	0.1 „	„	tr.
6.	0.05 „	„	n.

Both suspensions, but especially carcinoma emulsion retained a considerable quantity of the fixed amboceptors and gave to the goat blood cells some of them only. In other words, carcinoma cells fixed amboceptors from carcinoma serum more permanently than guinea-pig kidney did.



## 2. Transgression experiment with guinea-pig kidney serum.

Here the cell sediments obtained in fixation experiment 2 as shown in the following protocol were used:

0.2 c.c. (200 A.U.) guinea-pig kidney serum + 2.0 c.c. guinea-pig kidney emulsion + 0.8 c.c. saline = 3.0 c.c.

0.2 c.c. (200 A.U.) guinea-pig kidney serum + 2.0 c.c. mouse carcinoma 199 emulsion + 0.8 c.c. saline = 3.0 c.c.

To the sediments was added saline to the original volume (3.0 c.c.). The haemolytic reaction after the transgression was tested in the same way as before.

TABLE VIII.

	Amount of cell suspension	Haemolysis after transgression with	
		Guinea-pig kidney	Mouse carcinoma
1.	1.0 c.c.	c.	c.
2.	0.5 „	„	„
3.	0.3 „	„	„
4.	0.2 „	m.	„
5.	0.1 „	sl.	n.c.
6.	0.05 „	tr.	m.

The amount of guinea-pig kidney suspension which caused complete haemolysis is 0.3 c.c., while that of carcinoma suspension is 0.2 c.c. Thus it is seen that the kidney cells have bound the amboceptors of the kidney serum more strongly than the carcinoma cells.

## 3. Transgression experiment with mouse sarcoma serum.

The sediments used were those obtained in fixation experiment 6, the following being the protocol:

2.0 c.c. (100 A.U.) mouse sarcoma serum 37 p. + 2.0 c.c. mouse carcinoma 199 emulsion = 4.0 c.c.

2.0 c.c. (100 A.U.) mouse sarcoma serum 37 p. + 2.0 c.c. guinea-pig kidney emulsion = 4.0 c.c.

The haemolytic reaction after transgression was carried out as before.

TABLE IX.

	Amount of cell suspension	Haemolysis after transgression with	
		Mouse carcinoma	Guinea-pig kidney
1.	1.0 c.c.	n.c.	n.c.
2.	0.5 „	m.—sl.	m.
3.	0.3 „	sl.	sl.
4.	0.2 „	tr.	tr.
5.	0.1 „	n.	n.
6.	0.05 „	n.	n.

Mouse carcinoma cells and kidney cells bound the amboceptors of mouse sarcoma serum 37 p. strongly and practically to the same degree—1.0 c.c. of the suspensions not causing complete haemolysis.

## CONCLUSIONS.

1. Mouse carcinoma cells fix the haemolytic amboceptors of homologous serum more strongly than guinea-pig kidney cells.

2. Guinea-pig kidney cells fix the amboceptors of guinea-pig kidney antiserum more strongly than mouse carcinoma cells.

3. Goat blood cells are capable of fixing the amboceptors of both sera particularly well.

4. However, while goat blood cells can naturally absorb goat haemolysin, neither carcinoma nor kidney cells have any power of fixing goat haemolysin practically at all.

5. Rabbit and rat kidney cells, as they do not contain the lysinogen, do not absorb haemolytic amboceptors from the three antisera.

6. This fixation reaction is therefore to be regarded as specific.

7. Transgression experiments show that homologous antigens bind their amboceptors more strongly than heterologous; thus in all probability there is no complete identity between the haemolytic amboceptors which are produced by immunising rabbit against mouse tumours—carcinoma and sarcoma—and guinea-pig kidney. It will be remembered that Morgenroth and Bieling could not establish identity between carcinoma and mouse kidney haemolysis.

In conclusion, I desire to express my thanks to Dr Schütze for his advice in the course of this work.

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## A CONTRIBUTION TO THE STUDY OF AMBOCEPTORS AND RECEPTORS.

### THIRD COMMUNICATION ON HETEROLOGOUS IMMUNITY TO MALIGNANT MOUSE TUMOURS.

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It must be due to an incidental distribution of receptors that guinea-pig organs and mouse tumours are able to elicit haemolysins for goat erythrocytes, when they are inoculated into rabbit, because antigens are generally found in common only in closely related substances. So, if this relation exists between them, there arises a further question, namely, whether they may not be able to evolve other mutual antibodies; in other words, to what degree they may have antigenic substances in common. I have already noted (1915) that mouse tumour antisera contain complement fixing antibodies, using extracts of mouse and alien tumours as antigens. Michaelis and Fleischmann (1906) reported that rabbit sera immunised against guinea-pig and mouse liver fixed complement in the presence of their homologous antigens, namely, extracts of the livers of guinea-pig and mouse. Later, Fleischmann and Davidsohn (1908) published an extended investigation upon the same subject. So, it is easy to suppose that mouse tumour, guinea-pig kidney and goat erythrocyte antisera contain complement fixing antibodies. Haemagglutination, also, enters into the question, because the sera mentioned above are all haemolytic for goat erythrocytes.

#### I. COMPLEMENT FIXATION.

The immune sera employed for these experiments are those which were used for a previous work, namely:

1. Mouse carcinoma 199 antiserum,
2. Mouse sarcoma 37 p. antiserum,
3. Guinea-pig kidney antiserum, and
4. Goat erythrocyte antiserum.

All are naturally haemolytic to goat blood cells, and in order to carry out the complement fixation reaction with these sera, it was necessary to eliminate the haemolytic amboceptors from them (goat erythrocytes being used in the haemolytic system), this being done as follows:

In the case of mouse tumour and guinea-pig kidney sera, goat red cells were added in the proportion of 0.5 c.c. goat red cell deposit obtained by washing with saline, to 1.0 c.c. of serum, but in the case of goat erythrocyte serum where this quantity was not sufficient ( $\Delta.U. = 0.001$ ) it was necessary to absorb twice with 1.0 c.c. of goat blood cells to 1.0 c.c. of the serum. For antigen, watery extracts of mouse carcinoma and guinea-pig kidney, and alcoholic extract of goat blood corpuscles were employed. To prepare alcoholic goat erythrocyte extract I used v. Dungern's method (1912).

The author made up 20 % alcoholic extract from human blood corpuscles in order to test complement fixation with cancer patient serum. As the amount to be used he suggested 0.4 c.c. of this extract after a dilution of 1 in 2 with normal saline. In the present case the same amount of alcoholic goat erythrocyte extract has been employed. Von Dungern's method is, however, not suitable for the preparation of mouse tumour extracts, owing to precipitates being produced in some quantity when dilution with saline takes place. Consequently, watery extracts were employed. As to the method of preparation, healthy tumours were removed from animals which had been bled out, were cut up and washed with normal saline, and 10 % emulsions by weight of the tumour cells were made in physiological saline. These emulsions, after being kept in the cold room for two days, were centrifuged and 0.4 c.c. of the supernatant fluid employed for the reaction—double the quantity having been found not to interfere with complete haemolysis. With guinea-pig kidney extract the same method exactly was applied, and the same quantity employed. In the haemolytic system, 0.5 c.c. of 5 % goat erythrocyte suspension prepared from the deposit was used. The complement dose was 0.5 c.c. of a 1 in 10 dilution of normal fresh guinea-pig serum.

The results were noted after the tubes had been at a temperature of 37° C. for two hours, the degrees of haemolysis being distinguished as follows:

c. = complete	sl. = slight
n.c. = nearly complete	tr. = trace
m. = marked	n. = none



As control, it is necessary to test for complement fixation with normal rabbit serum, using the extracts already mentioned as antigens. For this purpose, sera from several normal rabbits were taken and examined after treating with goat blood cells just as in the cases of mouse tumour and guinea-pig kidney antisera. The results obtained from three of the rabbits are those seen in Table I.

TABLE I.

*Complement fixation by normal rabbit serum with mouse carcinoma, guinea-pig kidney and goat erythrocyte extracts.*

	Inactivated normal rabbit serum	Mouse carcinoma extract 0.4 c.c.	Guinea-pig kidney extract 0.4 c.c.	Goat erythrocyte extract 0.4 c.c.
1.	0.1 c.c.	c.	c.	c.
2.	0.05 "	"	"	"
3.	0.025 "	"	"	"
4.	0.01 "	"	"	"

Two further rabbits, however, gave different results, being those shown in Table II.

TABLE II.

*Complement fixation by normal rabbit serum with mouse carcinoma, guinea-pig kidney and goat erythrocyte extracts.*

	Inactivated normal rabbit serum	Mouse carcinoma extract 0.4 c.c.	Guinea-pig kidney extract 0.4 c.c.	Goat erythrocyte extract 0.4 c.c.
1.	0.1 c.c.	m.	m.	c.
2.	0.05 "	n.c.	n.c.	"
3.	0.025 "	c.	c.	"
4.	0.01 "	"	"	"

As the tables show, normal rabbit serum either does not contain complement fixing antibodies or does so to a very small extent.

The reaction carried out with sera immunised against mouse tumour, guinea-pig kidney and goat blood corpuscles however gave the following results:

TABLE III.

*Complement fixation by goat erythrocyte antiserum with homologous antigen and guinea-pig kidney and mouse carcinoma extracts.*

	Goat erythrocyte antiserum	Homologous extract 0.4 c.c.	Mouse carcinoma extract 0.4 c.c.	Guinea-pig kidney extract 0.4 c.c.
1.	0.1 c.c.	n.	n.	n.
2.	0.05 "	"	"	"
3.	0.025 "	"	sl.	"
4.	0.01 "	sl.	m.	sl.
5.	0.005 "	m.	n.c.	m.
6.	0.0025 "	c.	c.	c.
7.	0.001 "	"	"	"

TABLE IV.

*Complement fixation by mouse carcinoma antiserum with homologous antigen and guinea-pig kidney and goat erythrocyte extracts.*

	Mouse carcinoma antiserum	Homologous extract 0·4 c.c.	Guinea-pig kidney extract 0·4 c.c.	Goat erythrocyte extract 0·4 c.c.
1.	0·1 c.c.	n.	n.	n.
2.	0·05 „	„	tr.	m.
3.	0·025 „	„	m.	c.
4.	0·01 „	„	c.	„
5.	0·005 „	m.	„	„
6.	0·0025 „	c.	„	„
7.	0·001 „	„	„	„

TABLE V.

*Complement fixation by guinea-pig kidney antiserum with homologous antigen and mouse carcinoma and goat erythrocyte extracts.*

	Guinea-pig kidney antiserum	Homologous extract 0·4 c.c.	Mouse carcinoma extract 0·4 c.c.	Goat erythrocyte extract 0·4 c.c.
1.	0·1 c.c.	n.	tr.	n.
2.	0·05 „	„	sl.	„
3.	0·025 „	„	m.	„
4.	0·01 „	„	n.c.	s.l.
5.	0·005 „	tr.	c.	m.
6.	0·0025 „	m.	„	c.
7.	0·001 „	c.	„	„

Complement fixation tests with these sera gave with all three extracts positive results, although the sera deviated complement most strongly with their homologous antigens. In the case of goat erythrocyte serum this last is least marked, possibly owing to the removal of a certain amount of the more specific complement fixing antibody during the absorption with red cells.

## II. HAEMAGGLUTINATION.

The question as to the relationship between haemolysis and haemagglutination does not seem settled yet. Ehrlich and Morgenroth (1913) who deny that haemagglutination is a prelude to haemolysis, as Bordet (1913) and Baumgarten (1913) believe, assert that both antibodies can coexist independently in the same antiserum.

Forssman (1911) has described haemagglutination on goat erythrocytes with guinea-pig kidney antiserum, but he did not give an exact account of his observations. It remained to be seen, therefore, if guinea-pig kidney and mouse tumour antisera contain haemagglutinins, as well as haemolysins, for goat erythrocytes.

The methods employed were as follows:

The immune sera were inactivated by heating in the incubator at 56° C. for half an hour. The 5 % suspension of red corpuscles was prepared in the same way as for the preceding experiments. To each of the test tubes containing diminishing amounts of inactivated immune serum made up to 1 c.c. with normal saline, 0.5 c.c. of 5 % erythrocyte suspension and 0.5 c.c. of normal saline were added. After incubation the tubes were placed at room temperature and the following morning the results were read, the degrees of haemagglutination being distinguished as follows:

+++ = very distinct	± = doubtful
++ = distinct	- = negative
+ = positive	

The normal rabbit sera used in the complement fixation tests served as control.

TABLE VI.

*Haemagglutination of goat erythrocytes by normal rabbit sera.*

	1 c.c. of serum diluted	5 per cent. goat blood corpuscles	Normal saline	Result
1.	1 : 5	0.5 c.c.	0.5 c.c.	-
2.	1 : 10	"	"	-
3.	1 : 20	"	"	-
4.	1 : 40	"	"	-

Normal rabbit serum is thus seen not to contain haemagglutinin for goat erythrocytes at all.

The following tables show the same reaction carried out with various goat red cells, guinea-pig kidney and mouse tumour antisera.

TABLE VII.

*Haemagglutination of homologous erythrocytes by goat erythrocyte antisera.*

	1 c.c. of serum diluted	5 % goat blood corpuscles	Normal saline	Dilution	Goat erythrocyte serum		
					No. 1 A. U. = 0.001	No. 2 A. U. = 0.001	No. 3 A. U. = 0.0025
1.	1 : 5	0.5 c.c.	0.5 c.c.	1 : 10	+++	+++	+++
2.	1 : 10	"	"	1 : 20	+++	+++	+++
3.	1 : 20	"	"	1 : 40	+++	+++	+++
4.	1 : 40	"	"	1 : 80	+++	+++	++
5.	1 : 80	"	"	1 : 160	+++	±	+
6.	1 : 160	"	"	1 : 320	++	-	-
7.	1 : 320	"	"	1 : 640	+	-	-
8.	1 : 640	"	"	1 : 1280	-	-	-

TABLE VIII.

*Haemagglutination of goat blood corpuscles by guinea-pig kidney antisera.*

	1 c.c. of serum diluted	5% goat blood corpuscles	Normal saline	Dilution	Guinea-pig kidney serum		
					No. 1 A. U. = 0.001	No. 2 A. U. = 0.005	No. 3 A. U. = 0.005
1.	1 : 5	0.5 c.c.	0.5 c.c.	1 : 10	—	—	—
2.	1 : 10	..	..	1 : 20	—	—	—
3.	1 : 20	..	..	1 : 40	—	—	—
4.	1 : 40	..	..	1 : 80	—	—	—
5.	1 : 80	..	..	1 : 160	—	—	—
6.	1 : 160	..	..	1 : 320	—	—	—
7.	1 : 320	..	..	1 : 640	—	—	—
8.	1 : 640	..	..	1 : 1280	—	—	—

TABLE IX.

*Haemagglutination of goat blood corpuscles by mouse tumour antisera.*

	1 c.c. of serum diluted	5% goat blood corpuscles	Normal saline	Dilution	Mouse carcinoma serum	Mouse sarcoma serum
					A. U. = 0.005	A. U. = 0.02
1.	1 : 5	0.5 c.c.	0.5 c.c.	1 : 10	—	—
2.	1 : 10	..	..	1 : 20	—	—
3.	1 : 20	..	..	1 : 40	—	—
4.	1 : 40	..	..	1 : 80	—	—
5.	1 : 80	..	..	1 : 160	—	—
6.	1 : 160	..	..	1 : 320	—	—
7.	1 : 320	..	..	1 : 640	—	—
8.	1 : 640	..	..	1 : 1280	—	—

The tables show that goat erythrocyte antiserum certainly contains a haemagglutinin for goat blood corpuscles but that guinea-pig kidney and mouse tumour antisera do not. In other words, the receptors which are able to produce haemagglutinin for goat blood cells are not distributed in guinea-pig kidney and mouse tumours. One must therefore conclude that as the antigens for haemagglutinins and haemolysins for the same blood cells do not always exist side by side, but can be found separated from one another, haemagglutination is not necessarily a prelude to haemolysis.

## SUMMARY.

By using watery extracts of mouse tumours and guinea-pig kidney, and alcoholic goat erythrocyte extract, it has been shown that mouse tumour, guinea-pig kidney and goat erythrocyte antisera contain complement fixing antibodies for all three extracts, although the reaction is most evident when the homologous antigens are employed.



Goat erythrocyte antiserum agglutinates goat blood corpuscles strongly while guinea-pig kidney and mouse tumour sera do not contain haemagglutinin at all. Haemagglutination is therefore not to be regarded as a prelude to haemolysis: the antibodies for both processes can exist independently of one another. It has thus been seen that mouse tumour, guinea-pig kidney and goat erythrocytes have in common the property of producing, when inoculated into the rabbit, besides haemolytic amboceptors, complement fixing antibodies, but receptors for goat haemagglutinins cannot be found in either guinea-pig kidney or mouse tumours.

For his advice and assistance in carrying out this work I desire to thank Dr Schütze.

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## TRYPANOSOMIASIS IN NORTHERN UGANDA.

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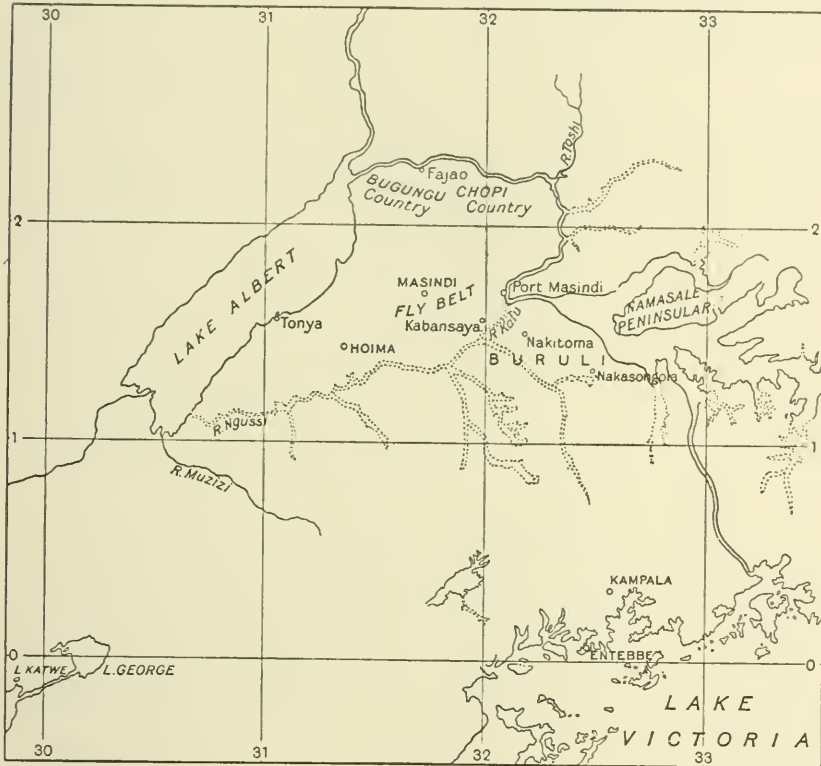
(With Map.)

IN January, 1914, Dr J. H. Reford, Uganda Medical Staff, reported the discovery, in the blood of a dog belonging to a European missionary, of a trypanosome showing marked morphological resemblance to the human parasite, the so-called *Trypanosome rhodesiense*. The dog had made a single journey through the *morsitans* belt south of Masindi. It died after a short and severe illness characterised by marked emaciation and keratitis. Dr Reford recognised a certain number of trypanosomes in which the nucleus was displaced posteriorly, and also sundry irregular, more or less rounded-off forms. Miss Robertson, who had just completed a tour in this same fly belt, examined some of Dr Reford's slides and expressed her agreement with his view as to the affinities of the organism to the Rhodesian trypanosome. As she had not met with any trypanosomes of the *gambiense-brucei* group during her experiments in the same district, Miss Robertson, in a report to the Principal Medical Officer dated 20th January, 1914, laid considerable stress on the importance of the find and discussed at some length the pros and cons of the arrival of the dreaded South African organism in this already overburdened Protectorate.

I was accordingly instructed to proceed to the Northern Province and thoroughly test the tentative hypothesis put forward by Miss Robertson. I arrived at Masindi in May, 1914, and continued in the district until September, 1914, when I was recalled for military service. The writing up of the experiments performed was unavoidably delayed until March, 1915, when I was enabled to prepare this report on the results obtained, while on temporary duty at Entebbe. There remains much to be done in this northern fly area, and I hope to renew my investigations at some future date.

During the latter part of 1913 Miss Robertson was engaged in the Northern Province on a series of investigations in connection with cattle trypanosomiasis in the Masindi *morsitans* region. A brief epitome of this work is here necessary, before considering the experiments which I subsequently carried out in the same district and which led me to somewhat different conclusions.

In June, July, and August, 1913, Miss Robertson visited and



examined the various scattered herds of cattle still remaining in the plains north and south of the Kafu river, and interrogated the natives regarding the past history of the Buruli-Kafu grazing grounds. At a spot on the Nakasongola-Kibangaya-Masindi Road (cf. Map), which traverses the fly belt, experiments were carried out. On no single occasion was any evidence obtained either in fly, experimental animals, or cattle, of the existence of a trypanosome of the *brucei* group. The fly experiments were carried out on the road where the mission dog

acquired its infection some three months later. Miss Robertson says however, "...but a survey of herds and the examination of a relatively small number of *Glossina morsitans*, such as I have made, cannot be considered a really exhaustive investigation of the country, and no subinoculations have been made of game."

During the reign of Kabalega, King of Bunyoro, the Buruli country, which at that time was a part of the Bunyoro kingdom, and the plains to the north of the Kafu supported large numbers of cattle. After the final defeat of the rebel king in 1895 he retreated to Bukedi across the Nile, and drove all his cattle away with him, practically clearing the country. About 1900-1901 cattle began to arrive from Buganda kingdom, and the old grazing grounds were restocked. In 1902-1903 disease began to show itself among the cattle in the Nakasongola district to the south, and slowly spread northwards to Nakitoma some two or three miles south of the Kafu river. "The disease was carried north of the Kafu by the moving of a single infected herd from Nakitoma in Buruli to Kibangaya in the Northern Province<sup>1</sup>." After this event "the sick animals in this herd died of their disease in about six months, and there was no further illness until about June, 1908, when all the herds in the Kafu district showed the disease one after the other. Now there is no reasonable doubt that Kabosolita's herd pasturing in country full of game, with tsetses in small numbers in the open grass plains and in tremendous numbers in the Kakora bush country only four or five miles away, must have been the source of the trouble. The fact that there was an interval of nearly two years between the arrival of Kabosolita's herd and the general infection of the district shows that the country at Kibangaya was clean when Kabosolita's cattle were taken there; also the general history of all other herds confirms this." "...It is important to note that it took nearly two years to produce an efficient endemic reservoir in the Kafu district" (north of Kafu)<sup>2</sup>; "moreover, owing to mutual relations of the game and the *morsitans*, the short period of six years has brought about a 10 % infection of the fly throughout the Masindi fly belt, even in places 10-12 miles from domestic herds."

From the above, which, as already stated, is Miss Robertson's account, the words in quotation marks being extracted from her reports, it is quite evident that she considers the cattle trypanosomes of the

<sup>1</sup> *Tropical Diseases Bulletin*, III. 419. Section 3, Miss Robertson's Report, September, 1913.

<sup>2</sup> My brackets.



Buruli and Kafu country, as well as those of the *morsitans* there found, were originally derived from the south.

As regards the trypanosome found by Dr Reford, Miss Robertson adopted a similar explanation of its presence in the *morsitans* of the Masindi belt. Her reasons were briefly as follows: during June, July and August, 1913, she was touring the district examining cattle and endeavouring to trace the aetiology and sources of the trypanosomiasis which had worked such havoc among the stock. She performed a series of experiments with *morsitans* caught on the very road where the mission dog became infected with the *brucei*-like organism in December, 1913.

In the course of these experiments 450 flies were fed on a monkey and a goat. The monkey developed *T. pecorum* "but never showed any infection with a member of the *gambiense-rhodesiense* group." The goat died of a mixed infection of *T. pecorum*, *T. vivax* and *T. uniforme*. Of these flies, 445 were dissected and 9.4 % found to contain developmental stages of various trypanosomes. "Proboscis only," "proboscis and gut" and "gut only" infections were noted; *i.e.* as will be seen later, none of these 445 flies harboured the full developmental cycle of the *brucei*-like organism described by Dr Reford.

Further 718 cattle were examined in various parts of the grazing area north of the Kafu without any sign of this latter organism being found, although 12 % were infected with trypanosomes.

The mission dog acquired its infection in December, 1913, *i.e.* some three months after the completion of Miss Robertson's experiments. In her report on the blood slide from the dog, sent from Masindi by Dr Reford, she says "it seems probable that the trypanosome which has appeared in the dog was not present in a sufficiently high percentage of the flies in the *morsitans* belt in July, 1913, to be detected by the methods adopted in the investigation. From the fact, however, that the dog passed apparently only once through the belt one is inclined to conclude, in spite of the element of chance which enters to a certain extent, that the trypanosome in question is now present in a greater proportion of the flies than in July, 1913. The deduction being that the trypanosome has been recently introduced into the *morsitans* belt" (Miss Robertson's report, 20th January, 1914).

It will be seen therefore that, according to Miss Robertson, both the trypanosomes responsible for the disease in cattle and this free-flagellated *brucei*-like organism have been imported from outside, and have secondarily established themselves in the *morsitans* belt near

Masindi: the reservoir being the wild game of the district. In other words the fly and game of this belt were presumably 'clean' say 20 years ago, and have reached their present degree of infectivity entirely as a result of a relatively recent introduction of trypanosomes from outlying parts of the Protectorate, in the case of the cattle parasites probably from the south.

I will now put forward some criticisms of this view, based partly on an exhaustive enquiry among the natives, with and without interpreters, supplemented by a series of questions put at my request to the Lukiko or native parliament of Bunyoro kingdom by the District Commissioners of Masindi and Hoima Stations; partly on my own experiments and observations.

First and foremost, as a general principle, the idea of a large area of very sparsely populated bush country, thick with game and *morsitans*, being free from trypanosomes is, I consider, untenable.

I hold the opinion that the pathogenic trypanosomes of cattle and domestic animals were originally derived from the apparently harmless parasites of wild game, the ultimate origin of these game trypanosomes being, of course, outside the range of the present discussion. It is curious to note, in various parts of Africa, that native owners often avoid grazing their cattle in the proximity of waterbuck and other scrub-feeding game because these animals are supposed to infect the grass with their saliva and other excretions. These same natives have no fear of the tsetse as such, although the results are the same as regards minimising the exposure of the cattle to disease.

In reviewing Miss Robertson's conclusions two criticisms at once suggest themselves.

(1) Disease or death in cattle in the area visited is assumed to be invariably due to trypanosomes, whereas in reality several other diseases are known to have occurred among these herds. The acting C.V.O., Mr V. F. Richardson, writes "with regard to native names and descriptions of disease, I should like to point out that these are very misleading. I have not found Bahima herdsmen able to distinguish rinderpest from trypanosomiasis, whilst the name *Nsotoka* appears originally to have referred to pleuro-pneumonia and is now used for rinderpest or acute trypanosomiasis. Even *Amakebbe*, the best known native name for a disease of cattle, is of doubtful significance, and I have known it applied to rinderpest and to undoubted cases of trypanosomiasis."

*Nsotoka* was one of the diseases described by the herdsmen in connection with the recent epidemic.

Again, Mr Hutchins, the C.V.O., Uganda Protectorate, on being consulted as to the probable diagnosis of the symptoms shown by the diseased cattle during the epidemic as supplied by various herdsmen around Masindi, expresses the opinion that some of these accounts point to *Babesia* as the causative agent.

(2) Miss Robertson is inclined to assume that once an animal is infected with trypanosomes it is doomed to a more or less speedy death. In the case of such trypanosomes as *T. nanum*, *T. vivax* and *T. uniforme* the result is not necessarily a fatal one, or, at any rate, not rapidly fatal. Both Kleine and myself have drawn attention to the extreme chronicity which often characterises infections with these parasites. Dr Marshall, early in 1914, found two of the animals in which Miss Robertson found trypanosomes in June-July, 1913, still alive and in excellent condition. Thus the conclusion that an animal has been recently infected because the only sign of disease is the occurrence of *T. vivax* or *T. nanum* in the peripheral blood is, I consider, not justifiable. Such an animal might have been infected for 12 months or more. With the more pathogenic trypanosomes the case is of course different, but the distinction is of importance in considering the history of an epidemic in relation to recent events.

As regards the native names for cattle diseases, the C.V.O. confirms my opinion that when the herdsmen refer to the disease *Lwakipumpuru* they mean genuine trypanosomiasis. Now it is interesting to note that, according to native testimony, there have been at least two epidemics of *Lwakipumpuru* in the Buruli cattle country before the one under discussion. Both these epidemics caused a very serious mortality among the cattle, and occurred respectively in the reign of Kabalega's father Kamrasi, say about 1860, and when the present King Anderea was a young boy, *i.e.* about 1890. *Lwakipumpuru* also played an important part in the recent epidemic. After each epidemic the Buruli country has been restocked with cattle, at longer or shorter intervals, from different sources, Buganda, Busoga and especially Bukedi. When Kabalega and his Banyoro were driven out of Buruli in 1895, they drove off all their cattle to Bukedi, to the neighbourhood of the Toshi river, a district thickly infested with *G. morsitans*. The majority of these cattle were brought back to Buruli by the victorious Buganda and re-established in their old grazing grounds. From what is now known of the Toshi river country, it is certain that a large percentage of these animals were infected with trypanosomiasis. Now *G. morsitans*, though found in the country south of the Kafu, is very



sparsely distributed. Nothing like the numbers found in the Masindi fly belt, north of the Kafu, occur in northern Buruli. The great tsetse reservoir is separated from these grazing grounds by the Kafu river and a belt of short grass plains some 8-10 miles wide—under ordinary circumstances an almost insuperable barrier to the fly. The Bukedi trypanosomes, under the fly conditions normally existing in Buruli, might be expected to die out with their original hosts. But given some particularly favourable year or season in which *morsitans* for some reason or other multiplied and spread, then we have all the requirements for an epidemic in Buruli. It is highly probable that such favourable seasons do occur from time to time, and *morsitans* become much more numerous in Buruli than they are at present. In the face of such an epidemic the cattle would be driven northwards across the Kafu, and the probability of their encroaching on the great Masindi fly belt would be increased. Again, with an increased number of cattle on the narrow strip of plain between the river and the fly country, there would be an increased likelihood of 'following fly,' accompanying traffic along the roads through the fly belt, meeting herds.

The part played by 'following fly' in the spread of disease is a very important one, as the following experiment arranged by Mr Fiske and myself shows. Two cyclists started from the plain near the Kafu at about 7.30 a.m. in bright sunshine, and rode some miles into the Masindi fly belt along two of the main roads. Turning round they then rode rapidly back to the ford where both roads cross the Kafu. Here, as each arrived, a canoe took him across to the Buruli side, and he walked his bicycle some 200 yards to where fly boys were awaiting him. In this way 16 *G. morsitans* were conducted across the river to a point some five or six miles from their home, across absolutely bare plains—and, curiously enough, on dissection five of these flies contained developmental stages of trypanosomes. No one who has cycled through *morsitans* country can have failed to be struck by the number of fly which follow the machine, and the distance they will travel. Mr Fiske tells me that one accompanied him on his bicycle as far as Masindi Station, which is some 12 miles from the northern edge of the fly belt. A slow pedestrian will soon shake off 'following fly,' but the pace of the cyclist seems to exert an irresistible attraction, and large numbers will follow for miles. Such agencies doubtless play their part in the spread of trypanosomiasis, and I consider it possible that the introduction of bicycles among the natives of Busindi, dating from 1908, had a definite influence on the recent epidemic.



The great fly belt which forms the northern limit of the Buruli-Kafu cattle country I regard as the principal source of all the trypanosome species found in the cattle, particularly those grazing north of the river. It is a permanent source, merely awaiting any condition which facilitates frequent exposure of the animal to fly bite. From this northern belt trypanosomes may be taken into central Buruli by

(1) 'Following fly' either with elephant, buffalo or other game which can easily cross the Kafu, especially in the dry season, or by cyclists and other traffic.

(2) By the passage of infected animals, game or cattle.

Other sources of trypanosomes in southern Buruli are afforded by the small *morsitans* foci which undoubtedly exist in that country, probably especially in association with the buffalo in the eastern part of the country; and also by the intermittent introduction of infected Bukedi or Busoga cattle.

It must be remembered that a native owner moves his herds directly he detects the presence of serious disease, and, as such movements are indiscriminate, he may well make matters worse by increasing their exposure to fly. The introduction of an infected herd into another region where the stock is apparently healthy, will expose these latter animals to the chance of direct transmission by biting flies other than *Glossinae*.

#### FLY EXPERIMENTS IN THE MASINDI MORSITANS BELT.

1857 wild *G. morsitans*, caught at various places in the fly belt south of Masindi, were fed upon suitable animals, more especially monkeys, and the trypanosome which, for convenience I will refer to as "*T. brucei*," appeared on eleven occasions. This gives a percentage of 0.59 % wild *morsitans* infected with this trypanosome. The figure is of little value, however, because the numbers of flies used for each experiment were unnecessarily large. In all, 13 experiments were performed, and only two of these failed to produce the long trypanosome; these two experiments involved 48 and 44 flies respectively. The above feedings were carried out from May 14th, 1914, to June 14th, 1914, *i.e.* approximately one year after Miss Robertson's visit.

*Trypanosoma pecorum*, *nanum*, *vivax* and *uniforme* were recovered on numerous occasions during these experiments, double and treble infections in the experimental animal often resulting.

During my stay in the Masindi belt, I came across two 'natural' infections in domestic animals which had been exposed to fly:

(a) A 'clean' dog, bought at Masindi and kept and examined at the laboratory there, sent to me along the road where the original mission dog became infected. This animal emerged from the fly belt at about 8 a.m. having commenced the journey during the night. It only made one passage, and five days later developed "*T. brucei*."

(b) A dog kept by Mr Fiske at his camp in the fly belt, which died of a pure *T. pecorum* infection.

The following figures show the results obtained by fly dissection performed in connection with the above feeding experiments. In interpreting the figures it must be borne in mind that (a) infections of proboscis only is ascribable to *T. vivax* or *T. uniforme*; (b) those of the proboscis and gut to *T. nanum* or *T. pecorum*; (c) those of the gut and salivary glands to the *brucei*-like organisms; those of the gut only to the immature infections with (b) or (c). There is no reason to suppose that any other pathogenic trypanosome than those mentioned occurs in this district.

A. Total of 1117 flies caught during experiments in fly belt, May and June, 1914:

13.4 % infected with trypanosomes,  
8.5 % proboscis only,  
3.1 % proboscis and gut,  
1.8 % gut not proboscis (three of these flies showed infected salivary glands).

B. With 264 of these flies caught within possible range of Miss Robertson's fly boys (*i.e.* around the place where her experiments were performed):

9.7 % infected with trypanosomes,  
6.8 % proboscis only,  
2.2 % proboscis and gut,  
0.7 % gut not proboscis (no infected salivary glands).

C. With 715 flies caught at places where waterbuck are not found or are very rare visitors:

9.6 % infected with trypanosomes,  
6.3 % proboscis only,  
1.8 % proboscis and gut,  
1.5 % gut not proboscis (1 fly showed infected salivary glands).

D. With 198 flies caught in a waterbuck locality:

25.2 % infected with trypanosomes,  
20.2 % proboscis only,  
4.0 % proboscis and gut,  
1.0 % gut not proboscis (1 fly had infected salivary glands).

E. With 206 flies caught in a second waterbuck locality:

15.8 % infected with trypanosomes,  
4.8 % proboscis only,  
6.7 % proboscis and gut,  
4.3 % gut not proboscis (1 fly showed infected salivary glands).

Miss Robertson's figures for July and August, 1913, given for comparison, are

445 flies dissected 9.4 % infected with trypanosomes,  
3.8 % proboscis only,  
2.9 % proboscis and gut,  
2.7 % gut, not proboscis

(no flies found with infected salivary glands).

These figures are very significant in reference to Miss Robertson's opinion that the *T. brucei* is a new arrival in these Masindi *morsitans*. If this *T. brucei* were a new introduction it would be reasonable to expect an increase in the 'gut not proboscis' figures in one or both of Sections A and B of my figures.

It is interesting also, as pointing to the presence of this trypanosome in the *morsitans* of the Masindi belt before Miss Robertson's arrival, that the native dogs were dying in 1912-13 with symptoms of keratitis. This symptom is almost always due to *T. brucei*, and rarely if ever to the *T. pecorum* present in these fly.

In the following fly-feeding experiments the results were controlled by dissection of all the flies employed as they died, either during the experiment or at its conclusion.

It will be seen that on every occasion where a positive salivary gland was seen, the animal fed upon developed "*T. brucei*"; also that *T. nanum* appears to be more common in these wild *morsitans* than *T. pecorum*.

*Trypanosomiasis in Uganda*

Animal fed upon	Number of flies fed	Positive flies					Trypanosome species appearing in animal fed upon	Remarks
		Number found during experiment	Site of trypanosomes					
			Proboscis only	Proboscis and gut	Gut and salivary glands	Gut only		
Goat D	28	5	3	1	0	1*	" <i>T. brucei</i> ," <i>vivax</i> and <i>pecorum</i> or <i>nanum</i>	Only a small piece of the salivary gland of * fly seen
„ C	48	5	0	2	1	2	" <i>brucei</i> ," and <i>nanum</i>	Subinoculation from goat into dog excluded <i>pecorum</i>
Sheep A	48	11	9	2	0	0	<i>vivax</i> and <i>nanum</i>	Subinoculation from sheep into dog excluded <i>pecorum</i>
Goat E	44	4	1	1	0	2	<i>vivax</i>	
Monkey K	32	9	7	1	1	0	" <i>brucei</i> "	Injection of salivary gland into monkey produced " <i>brucei</i> "
„ P	64	9	5	3	1	0	" <i>brucei</i> "	
„ Q	46	9	8	1	0	0	nil	
„ H	27	9	8	1	0	0	nil	

The following inoculations were made of the blood of game shot in and around the fly belt into clean experimental animals:

Game species inoculated	Number injected	Number + on microscopic examination	Nature of infection in game; single or mixed	Species of organism appearing in sub-inoculated animals
Hartebeest	7	1	single	" <i>brucei</i> "
Ugand cob	7	0	—	0
Reedbuck	1	0	—	0
Bushbuck	5	2	single	<i>uniforme</i> ; <i>pecorum</i>
Warthog	1	0	—	0
Buffalo	4	0	—	0
Duiker	2	0	—	0
Waterbuck	3	2	mixed; simple	<i>nanum</i> ; <i>vivax</i> ; " <i>brucei</i> "
Totals	30	5	—	0

This gives a total infected with trypanosomes of 16.6 %: 6.6 % carried "*T. brucei*."

Experiments in other tsetse districts of the Northern Province to determine the distribution of the *brucei*-like organism:

#### 1. NGUSSI RIVER, 3-4 MILES FROM THE FALLS (cf. MAP).

In this neighbourhood three species of *Glossina* were obtained, *G. pallidipes*, *G. palpalis*, and *G. fusca*.

Fly dissections:

*G. pallidipes*. 65 flies dissected.

18.4 % infected with trypanosomes,

1.5 % proboscis only,



7.6 % gut only,  
6.3 % proboscis and gut,  
3.0 % gut and salivary glands.

*G. palpalis*. 95 flies dissected.

8.4 % infected with trypanosomes,  
2.2 % proboscis only,  
1.0 % gut only,  
5.2 % proboscis and gut.

No salivary gland infections seen.

*G. fusca*. 5 dissected—all negative.

Fly feeding experiments:

*Dog X*, fed upon by 110 flies, a mixture of *pallidipes* and *palpalis*, developed an uncontaminated "*T. brucei*" infection. Dissection of these flies revealed two with the salivary glands swarming with trypanosomes, and several flies with 'proboscis' and 'proboscis and gut' infections.

*Dog Y*, fed upon by a mixture of 48 *G. palpalis* and *G. pallidipes*, the majority *palpalis*, remained clean. Dissection of the flies revealed several 'proboscis' and 'proboscis and gut' infections, but no sign of any infected salivary glands.

Here again a positive salivary gland leads to infection with "*T. brucei*"; while the fact that the majority of the 'proboscis and gut' infected flies had obviously fed on the dogs without producing infection indicates that, here also, *T. nanum* is commoner than *T. pecorum*.

I have already pointed out (*Sleeping Sickness Report*, XII, 7, and XIII, 5, and *Proc. Roy. Soc. B.* vol. LXXXV, 1912) that *T. nanum* is better adapted to development in and transmission by *G. palpalis* than is *T. pecorum*, and these figures point to the same conclusion.

No game inoculations were made in this district.

## 2. REGION OF THE TONYA PENINSULA AND SHORE OF LAKE ALBERT.

Tsetse species found, *G. palpalis*.

407 flies dissected: 9.9 % infected with trypanosomes,  
2.7 % proboscis only,  
1.4 % proboscis and gut,  
5.8 % gut only.

A considerable proportion of these 'gut only' flies contained *T. grayi*. No salivary gland infections were found.

## Game inoculations:

Game injected	Number infected on microscopic examination	Number injected	Infection in game : single or mixed	Trypanosome recovered
Buffalo	0	4		
Bushbuck	2	9	single	<i>nanum</i>
Waterbuck	0	2		
Duiker	0	3		
Cob	0	1		
Warthog	0	1		
Lion	0	2		
Totals	2	22		

i.e. 9 % infected with *T. nanum* which is here carried by *G. palpalis*.

## Fly feeding experiments:

*Goat F*, fed upon by 393 *palpalis*, developed *T. nanum* or *T. uniforme*.

*Goat G*, fed upon by 570 *palpalis*, developed *T. nanum* or *T. uniforme*.

*Goat H*, fed upon by 370 *palpalis*, developed *T. nanum* or *T. uniforme*.

## 3. CHOPI, VICTORIA NILE AND BUGUNGU REGION.

Fly dissections: *G. palpalis* caught around Foweira on Victoria Nile.

154 flies dissected: 6.4 % infected with trypanosomes,  
3.2 % proboscis only,  
0.00 % proboscis and gut,  
3.2 % gut only.

A large proportion of these 'gut only' flies carried *T. grayi*.

No game was obtained here.

*G. palpalis* caught on Bugungu plain.

57 flies dissected: 8.7 % infected with trypanosomes,  
7.0 % proboscis only,  
0.00 % proboscis and gut,  
1.7 % gut only.

A proportion of these 'gut only' flies carried *T. grayi*.

*G. morsitans* caught in Bugungu and around Fajao.

606 dissected: 9.6 % infected with trypanosomes,  
7.0 % proboscis only,  
1.8 % proboscis and gut,  
0.8 % gut only.

No *T. grayi* seen.

*Fly breeding experiments with fly caught in Bugungu country and around Fajao.*

Dog M, 218 *G. palpalis*, no trypanosomes recovered.

Dog N, 520 *G. morsitans*, developed *T. pecorum*.

Goat X, 760 *G. morsitans*, developed a mixed infection with *T. vivax*, "*T. brucei*," and a *pecorum-nanum* type of organism.

Monkey L, 440 *G. morsitans*, died on seventh day after last batch of flies had fed, having no trypanosomes in its blood.

*Injection of blood of game shot around Fajao and in Bugungu country.*

Game injected	Number injected	Number + on microscopic examination	Infection in game	Trypanosome
Waterbuck	9	2	—	<i>uniforme</i> or <i>nanum</i>
Bushbuck	4	2	mixed	<i>nanum</i> and <i>vivax</i>
Elephant	1	0		
Buffalo	1	0		
Cob	3	0		
Hartebeest	1	0		
Warthog	1	0		
Totals	20	4		

Total infected with trypanosomes, 19 %.

#### 4. NAMASALE PENINSULA.

No tsetse seen.

Blood of following game was injected and examined: hartebeest 3, cob 3, warthog 1, reedbuck 1, rhino 1, elephant 1 = total of 10 animals. No trypanosomes were seen.

#### 5. NORTHERN BURULI.

No tsetse seen.

Blood of following game was inoculated and examined without any trypanosomes being seen: hartebeest 6, eland 2, cob 5, bushbuck 2, waterbuck 2 = 17 animals.

The animal reactions and morphology of all the strains of the "*T. brucei*" which I recovered during the above investigations were such as to warrant the conclusion that they belong to the same species. The disease is acute in dogs and is characterised by keratitis in the great

majority of cases. Oedema of the face is often present in sheep and goats. One striking exception to the rapid course usually pursued by infections with this trypanosome was afforded by a young monkey which was infected by wild fly on May 30, 1914, and was still alive and active on Sept. 11, 1914, though intermittently showing trypanosomes in its blood.

The average duration of the disease in eight experimental dogs was 26 days, and in five monkeys 40 days; the young monkey mentioned above was not included in estimating this average. In seven out of the eight dogs, keratitis intervened. It is difficult to estimate the course of the disease in sheep and goats as no sub-inoculations were made into these animals; and in every instance where "*T. brucei*" developed as a result of feeding wild fly upon them, mixed infection resulted.

The above facts show that a trypanosome of the *brucei* group is widely distributed throughout the southern part of the Northern Province, probably, indeed, wherever *G. morsitans* and *pallidipes* are found. Thus it has been recovered from the Ngussi river to the south-west, from Bugungu and Chopi to the north, in addition to being found throughout the main belt south of Masindi station. Further, Dr Bagshawe, in his report for December, 1906, alludes to the occurrence of a similar organism on the Muzizi river at the southern end of Lake Albert, and also in the neighbourhood of Lake George. Dr Hodges, Principal Medical Officer, Uganda Protectorate, described a similar trypanosomiasis in the neighbourhood of Gondokoro in 1904-5, in which oedema of the face and keratitis were prominent symptoms, and which strongly suggests the presence of the same or a similar organism. In *Sleeping Sickness Report*, XIV, 2, I described a trypanosome from fly and game recovered from the Katwe region of Toro Province, and Lake George.

Speaking generally, wherever cattle are exposed to the bites of tsetse, especially of *morsitans* and *pallidipes*, they sooner or later sicken and die out. No one, so far as I am aware, has yet reported the existence of a locality where cattle are tolerant to the game tsetses.

It would appear then that this, at first sight, alarming discovery in the Masindi fly belt of a trypanosome showing close affinity to the organism recently isolated from man in South Africa need not cause any undue alarm. A similar or identical trypanosome will probably be found in every *morsitans* or *pallidipes* area in Africa. Here and there, for what reasons we know not, it may, as in Rhodesia, develop



the faculty of more or less permanent survival in man, a host normally immune. As such it must be viewed as a potential source of danger to human beings<sup>1</sup>. As regards the local aspect of the question, I visited nearly all of the few scattered villages in the fly belt and examined as many of the natives as possible. In this way 288 natives were seen and examined by gland palpation with negative result. There was no sign of any form of human trypanosomiasis, acute or chronic. The actual figures were

Adult males	131
Adult females	135
Children	22
Total	288

These figures, though small, none the less represent a considerable proportion of the inhabitants of the actual fly belt.

As regards the nomenclature of this organism and its full significance to the Protectorate, I cannot at present pronounce a final opinion. Experiments had been devised for further research on this interesting subject, but they had to be suspended owing to the outbreak of war.

Meantime, from an administrative point of view, I do not consider it to be a human parasite. The natives examined were selected from villages within the fly belt, and *morsitans* were to be seen actually in their villages. These fly have been shown to be infected with the *brucei* organism in the proportion of 0.5 %. The percentage varies in different parts of the belt, the difference apparently having some relation to the distribution of game. These people must be bitten many times a day. Domestic animals cannot survive; dogs die with keratitis and emaciation, and an occasional goat, all one finds in these scrub villages.

From a theoretical and scientific point of view, we must admit that the presence of a trypanosome so nearly allied to the human parasite of Rhodesia constitutes a potential source of danger. Practically, however, provided that steps are taken against the introduction of a large number into the at present sparsely populated fly area, there is no reason to expect any untoward developments.

<sup>1</sup> Cf. *Journ. Trop. Med. and Hyg.* June 15, 1915.

## AN IMPROVED METHOD FOR THE CONCENTRATION OF ANTITOXIC SERA.

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GIBSON (1905) was the first to introduce a method of commercial value for the concentration of antitoxic horse sera.

He treated plasma with an equal volume of saturated ammonium sulphate solution, filtered off the precipitated globulins and suspended the latter in saturated salt solution. The pseudoglobulin and antitoxin entered into solution while the euglobulin remained in suspension. The solution of pseudoglobulin and antitoxin was freed from euglobulin by filtration and the clear filtrate was then acidified with acetic acid. The precipitated pseudoglobulin and antitoxin were dialysed. The finished product had a potency two and a half times greater than that of the original plasma.

In 1907 Banzhaf and Gibson, by fractional precipitation with ammonium sulphate, separated the pseudoglobulin-antitoxin combination from the other serum proteins as follows:

The plasma or serum, diluted with one-third its volume of water, was heated to a temperature of 57° C. for 15 hours or of 58° C. for 7 hours. The liquid was then made 30 % saturation with ammonium sulphate<sup>1</sup> and the ensuing precipitate (Fraction 1) separated by filtration.

The Fraction 1 precipitate was suspended in saturated brine. The brined liquid was filtered from the insoluble euglobulin and the filtrate, containing in solution the pseudoglobulin and antitoxin which had been present in the Fraction 1 precipitate, was made 0.25 % with glacial acetic acid. The ensuing precipitate was filtered, pressed and dialysed.

<sup>1</sup> The phrase "made  $x$  % saturation with ammonium sulphate" has been used to indicate the following: to 100- $x$  parts of the serum by volume there was added  $x$  parts by volume of a saturated aqueous solution of ammonium sulphate.

The filtrate from Fraction 1 was made 54 % of saturation with ammonium sulphate and the precipitate (Fraction 2) thus formed was filtered, pressed and dialysed. The residue from the dialysis of the second Fraction precipitate is the more potent and may be used separately. In practice the dialysis residues from Fractions 1 and 2 were usually mixed and the requisite amount of salt and preservative was added. The finished product by this method had a protein content of about 18 to 20 % and a unitage per c.c. about 4 to 5 times greater than that of the original plasma.

In 1912-13 Banzhaf introduced his One Fraction method in which serum or plasma, diluted with half its volume of water and made 30 % of saturation with ammonium sulphate, is brought up to a temperature of 60° C. and kept at that temperature for a few minutes only, the entire heating process taking about 2 hours in all.

The hot serum mixture is then filtered. The precipitate of euglobulin is well pressed or is washed with 33 % saturation with ammonium sulphate; the washings are filtered and added to the main bulk of the filtrate which is then made 50 % of saturation with ammonium sulphate. The ensuing precipitate, consisting of pseudoglobulin and antitoxin, is filtered, pressed and dialysed. The dialysis residues are treated in the same way as in the Banzhaf-Gibson process.

The protein content of the finished product is about 20 % while the unitage per c.c. is 4 to 5 times that of the original serum.

Banzhaf's new method certainly possesses the following advantages over the former methods:

- (1) There is only one finished product for dialysis.
- (2) There is a considerable saving of time as the lengthy process of extracting the first fraction precipitate with brine is obviated.
- (3) The expenses incurred in the process are considerably less, both as regards labour and the use of precipitating materials, etc.

But unfortunately practical experience shows that the method is not always easy to work as the production of a readily filterable end product which will remain clear is dependent upon the condition of the serum mixtures after the preliminary heating process. If the latter do not filter rapidly and easily the end product will not be satisfactory. In such cases it is necessary to allow the dialysis residues to stand in the ice chest for weeks so that the opalescent suspension therein may settle, otherwise filtration through filter candles is accomplished with great difficulty and the formation of a solid deposit in the antitoxin in its vial containers occurs.

This delay is a great disadvantage and nullifies any gain in time which should accrue from the fact that there is only one fraction to be considered.

In this laboratory we have ascertained that the irregularities in the filtration can be obviated by the addition of  $1\frac{1}{2}$  to 2 % of solid sodium chloride to the serum mixtures thus:

The serum or plasma is diluted with one-half its volume of water, made 30 or 31 % of saturation with ammonium sulphate and to the mixture is added  $1\frac{1}{2}$  to 2 % of solid sodium chloride. The temperature of the mixture is gradually raised to  $61^{\circ}$  or  $63^{\circ}$  C. and kept at the chosen temperature for not more than five minutes. The mixture is cooled to  $40^{\circ}$ – $45^{\circ}$  C. before filtration.

The filtration of the hot mixture and of the subsequent 50 % saturation mixture proceeds rapidly. Moreover the end product has, up to the present, proved satisfactory.

Banzhaf considers that in his new process the value of the heating lies in the conversion of soluble pseudoglobulin into a further amount of euglobulin and that the rapid filtration of the serum mixture depends upon the success of the operation. In a recent communication (1916), however, I have demonstrated that there is no appreciable conversion of soluble into insoluble protein under the heating conditions advocated in his One Fraction process. The value of the heating process lies in the complete aggregation of the particles of precipitated euglobulin into conglomerates of such dimensions that they are readily retained on the surface of filter paper. The particles of euglobulin not sufficiently aggregated to be retained by the filter paper will ultimately be an impediment to the filtration of the final product through filter candles. Any suspensoid particles of euglobulin passing through the filter candles will impart an opalescent appearance to the finished product, and will gradually be deposited.

Also, I drew attention to the variability of the precipitating power of 30 and 31 % of saturation with ammonium sulphate. It was noticed that with sera in which there had been a comparatively slight removal of protein by 30 or 31 % of saturation with ammonium sulphate, the heated serum mixtures without exception filtered badly. The addition of  $1\frac{1}{2}$  to 2 % of sodium chloride to the serum mixtures before heating remedied this defect.

From these and from other observations it was concluded that the reaction of the sera and of the serum mixtures must be taken into account before the Banzhaf New One Fraction method could be depended



on to furnish a permanently satisfactory end product. In this connection an investigation is being pursued as to whether the sodium chloride improves the filtration mainly by virtue of its specific action on globulins or by decreasing the hydrolysis of the phosphate of sodium to which the alkalinity is due. If the latter be the case then the desired improvement in filtration should be obtained by the addition to the medium of the amount of acid necessary to adjust the reaction to that required for optimum precipitation.

As the experimental work involved in this investigation must of necessity extend over a considerable period of time, I have, in the meantime, sought for some better method of removing from the serum mixture a relatively greater amount of protein without increasing the loss of antitoxin and without adding to the labour involved: the idea being to diminish as far as possible the amount of euglobulin in opalescent suspension in the final product.

The necessary clue was furnished by a study of the changes taking place in the solubility of serum proteins during the heating of serum or plasma (Table I). The changes may be summarised as follows:

(1) If serum or plasma be heated just to a temperature of  $61^{\circ}$  or  $63^{\circ}$  C. and kept at that temperature for a few minutes only, there is no appreciable change in the solubility of the serum proteins neither is there any change in the precipitating power of 30 % of saturation with ammonium sulphate. The heating process merely serves to completely aggregate the particles of precipitated euglobulin into a filterable form.

(2) If diluted serum or plasma be heated to a temperature of  $61^{\circ}$  or  $63^{\circ}$  C. and be kept at those temperatures for about 1 hour there is a considerable change, about 25 %, in the precipitating power of 30 % of saturation with ammonium sulphate. There is also a corresponding change if the serum or plasma be heated just to  $67^{\circ}$  (about 15 to 20 %).

(3) If serum or plasma diluted with one-third its volume of water be heated to a temperature of  $55^{\circ}$  C. for 15 hours there is only a slight change in the solubility of the serum proteins. But there is a 10 % increase in the precipitating power of 30 % saturation with ammonium sulphate.

(4) If diluted plasma or serum be heated to a temperature of  $56^{\circ}$ – $57^{\circ}$  for 15 hours, or to  $58^{\circ}$  for 7 to 8 hours, there is again only a slight change in the solubility of the serum proteins. But, the precipitating power of 30 % ammonium sulphate is increased from 30 to 49 %.

The changes in (4) are comparable with those observed by Banzhaf (1908). He regards them in the light of a conversion of pseudo- into

euglobulin, but in view of the work of Hardy (1899), Chick and Martin (1912) it is more probable that the changes taking place in (1), (2), (3) and (4) are of the nature of a heat denaturation of proteins.

In devising a new method for the concentration of antitoxic sera there were, in view of my experimental observations, the following factors to be borne in mind:

(1) The complete aggregation under the influence of heat of the suspensoid particles of precipitated euglobulin into conglomerates sufficiently large to be retained on the surface of filter paper.

(2) The heat denaturation of serum proteins whereby their precipitability by 30 % saturation with ammonium sulphate is considerably increased.

(3) The stimulating effect of the addition of 1.5 to 2 % of solid sodium chloride on the process in (2)<sup>1</sup>.

Experimental work was then undertaken in order to ascertain the most favourable conditions under which to ensure the denaturation of the proteins without appreciable loss of antitoxin. It was found that the more rapid denaturation induced by heating the serum or plasma at 61° or 63° C. for an hour was followed by an appreciable loss of antitoxin. With the prolonged heating of the serum or plasma at 55° C. there was a slight loss only of antitoxin but at the same time the denaturation of the proteins was only 10 %. At temperatures between 56°–58° C. there was, comparatively speaking, a slight loss only of antitoxic units while there was a considerable denaturation of protein.

#### *The technique adopted.*

The serum or plasma, diluted with one-third or even with only one-fifth its volume of water, and made 1.5 to 2 % with solid sodium chloride, is heated to a temperature of 56°–57° C. for 15 hours or to 57°–58° C. for 8 hours<sup>2</sup> (Stage I).

During the prolonged heating a considerable change in the precipitability of the proteins occurs (Tables II *a* and *b*).

<sup>1</sup> Our experiments so far are in favour of the adjustment of the medium by means of salt rather than by the addition of acid.

<sup>2</sup> We prefer to heat the serum or plasma at the lower temperature for the longer period. But, in the routine work it is sometimes more convenient to make arrangements for an 8 hours' than for a 15 hours' heating.

It is not really necessary to dilute the plasma or serum in order to ensure the required change during the prolonged heating, for the heat denaturation takes place to the same extent in the undiluted as in the diluted fluid. But in order to obtain the best conditions

The heated plasma is then made 30 % of saturation with ammonium sulphate and the mixture is heated to a temperature of 61° C. and kept at that temperature for a few minutes only (Stage II).

By raising the temperature of the serum-ammonium-sulphate mixtures to 61° the aggregation of the particles of suspensoid protein into a precipitate which can be readily separated by filtration through paper takes place without the precipitation of a further amount of soluble protein and without loss of antitoxin.

The mixture after cooling to 40°–45° C. is filtered. The precipitate is washed with 33 % of saturation with ammonium sulphate. The washings, after being filtered, are added to the main bulk of the filtrate which is then made 50 % of saturation with ammonium sulphate.

The resulting precipitate is filtered off, pressed and dialysed. The pressed precipitate has a yellowish colour and not the bluish green colour of Banzhaf's product.

To the residues from dialysis are added the necessary amounts of salt and preservative. The final products in bulk are of a reddish brown colour and do not exhibit even a trace of an opalescent suspension. In layers of about 1 to 2 inches thick the liquids are transparent and are of a yellowish brown colour.

The filtration of these products through pulp and through Pasteur-Chamberland filters, up to the time of writing, has presented no difficulty. Moreover the dilution of the dialysates with two or three times their bulk of 1 % saline does not impair their ease of filtration. In this respect alone the final product is superior to that obtained in the Banzhaf One Fraction process.

In the method thus advocated in this paper there have been incorporated the important factors in the Banzhaf-Gibson Two Fraction method, the Banzhaf One Fraction method and in my modification of the latter.

The method was carried out at first on an experimental scale and promised success: it was then used with a batch of 10 litres of oxalated plasma. It was found that, during the process the removal of about

for a satisfactory precipitation and subsequent heat aggregation of the protein precipitated by 30 % saturation with ammonium sulphate it is, in our opinion, necessary to make the above dilution with water before adding the necessary amount of ammonium sulphate solution.

So far as the denaturation without loss of antitoxin is concerned the same results may be obtained by making the plasma or serum 30 % of saturation with ammonium sulphate previous to Stage I of the heating process. But in this case the end product is not so satisfactory: it is distinctly opalescent.

70 % of the total proteins of the original plasma was effected, and at the same time, even though the precipitate from the 30 % saturation with ammonium sulphate had not been extracted, there was only a loss of 10 % of antitoxic units. The end product was so satisfactory that routine concentrations were carried out by this method.

Several batches of plasma and of serum, each of 100 litres, have been concentrated so far with excellent results both for tetanus and for diphtheria antitoxic sera. The details of typical concentrations carried out by this method and by the Banzhaf One Fraction process are given in Table III.

The denaturation of the serum proteins during the prolonged heating of the serum and their consequent increased precipitation by 30 % of saturation with ammonium sulphate has led to the preparation of a final product in which the protein content is about 17–19 % while the unitage per c.c. has been increased to 8–9 times that of the original serum. Moreover if the process be carried out with the customary precautions against undue loss and if all precipitates, filter papers, etc., used in the process be carefully washed with 33 % ammonium sulphate and added to the main filtrates from the first precipitate, the loss of antitoxic units need not be greater than 10 %; in fact it can be reduced to as little as 5 %.

Furthermore, the final product is a clear limpid fluid which, even on dilution with 2 or 3 parts of a 1 % salt solution, shows no trace of opalescence and therefore should remain permanently clear.

**The method given above is an improvement on**

*A. The Gibson-Banzhaf Method for four reasons.*

(1) There is only one fraction to consider and the extraction of the first precipitate with brine is no longer necessary. This involves the saving of time, labour and materials.

(2) The heating of the serum made 30 % of saturation with ammonium sulphate serves to agglutinate the suspensoid particles of protein into conglomerates sufficiently large to be retained by filter paper. In the Gibson-Banzhaf process, in order to ensure the retention of these particles, it was often necessary to increase the percentage of ammonium sulphate to 35.

(3) The potency of the finished product is nearly *twice* as great as that obtained by the Banzhaf-Gibson process while the protein content is only 17 to 19 %.

(4) The total loss of antitoxic units need not be greater than 10 %.



**And on***B. The Banzhaf One Fraction Process for three reasons.*

(1) By the removal of a much greater amount of protein, as a result of the heat denaturation of serum proteins and their consequent increased precipitability by 30 % of saturation with ammonium sulphate, there is less likelihood of filtration difficulties.

(2) By the addition of  $1\frac{1}{2}$  to 2 % of sodium chloride to the serum mixture there is an adjustment of the reaction of the medium towards the conditions required for more complete precipitation of the particular proteins thrown out of emulsoid solution by the 30 % saturation with ammonium sulphate. This factor has a beneficial effect on filtration.

(3) The finished product is nearly *twice* as potent per c.c. as that obtained by the concentration of the same sera by Banzhaf's One Fraction method without containing a higher percentage of protein.

**SUMMARY.**

The method for the concentration of antitoxic sera suggested in this communication and now being used in this laboratory, presents a further step towards the desired goal, viz. the preparation, on a commercial scale for general therapeutic use, of antitoxic sera with a minimal amount of attendant protein.

In the concentration of sera on a large scale by the routine methods hitherto published the final products have shown a protein content of about 18 to 20 % with a potency per c.c. four to five times that of the original serum. The above described method yields, as a matter of routine, a final product with a protein content of about 17 to 19 % and with a potency per c.c. as much as nine times that of the original serum. With the use of better processes the potency might be higher still.

It is worth while to concentrate by this method plasma or sera in which the unitage is so low that hitherto they would have been discarded as having too low a potency for use even after concentration.

The patient will receive a relatively higher percentage of antitoxic units per gram of protein than if the sera had been concentrated by other methods, for by this method the removal of 70 % of the total proteins is ensured.

The heat denaturation of the proteins during the prolonged heating of the serum and consequent diminution of protein relatively to antitoxin may serve to minimise troubles with serum sickness.

In conclusion I desire to express my thanks to Dr A. T. MacConkey for the interest he has taken in the work and for the facilities accorded to me for the furtherance of the investigation.

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TABLE I.

*The effect of the heating of serum or oxalated plasma (a) on the solubility of the serum proteins and (b) on their precipitability by 30 % of saturation with ammonium sulphate.*

	Percentage protein content of serum or plasma	Proportion of water added to the serum or plasma before heating	Temperature to which diluted serum or plasma was heated	Duration of the time of heating at the chosen temperature	(a)		(b)	
					Percentage of protein in solution, calculated from the Refractometer Readings and expressed in terms of the original serum or plasma	Percentage conversion of soluble protein into insoluble protein during the heating process	Percentage in the filtrates from the serum or plasma in (a) made 30 % of saturation with ammonium sulphate. Calculated from the Refractometer Readings and expressed in terms of the original plasma or serum	Percentage increase in the precipitation of protein by 30 % of saturation with ammonium sulphate
I	7.78 7.78	$\frac{1}{2}$ $\frac{1}{2}$	Room temperature 63° C.	5 minutes	7.78 7.52	— 3.3 %	6.11 5.92	3.0 % 3.0 %
II	6.49 6.49	$\frac{1}{2}$ $\frac{1}{2}$	Room temperature 63° C.	$\frac{3}{4}$ hr	— —	— —	5.76 4.28	24.0 %
III	6.23 6.23	$\frac{1}{2}$ $\frac{1}{2}$	Room temperature 67° C.	5 minutes	— —	— —	4.98 4.00	19.8 %
IV	7.39* 7.39	$\frac{1}{2}$ $\frac{1}{2}$	Room temperature 55° C.	15 hrs	7.39 6.89	— 6.7 %	5.35 4.81	10.0 %
V	7.38 7.38 7.38	$\frac{1}{2}$ $\frac{1}{2}$ $\frac{1}{2}$	Room temperature 56°-57° C. 56°-57° C.	4 hrs 8 hrs	7.38 7.05 7.01	— 4.5 % 5.0 %	5.12 — 4.82	5.5 %
VI	6.13 6.13 6.13	$\frac{1}{2}$ $\frac{1}{2}$ $\frac{1}{2}$	Room temperature 56°-57° C. 56°-57° C.	6 hrs 15 hrs	6.13 5.99 5.79	— 2.3 % 5.5 %	5.78 5.48 3.52	5.2 % 39.1 %
VII	7.10 7.10	$\frac{1}{2}$ $\frac{1}{2}$	Room temperature 58° C.	7-8 hrs	7.10 6.99	— 1.5 %	5.52 3.76	31.9 %
VIII	7.59* 7.59	$\frac{1}{2}$ $\frac{1}{2}$	Room temperature 58° C.	7-8 hrs	— —	— —	4.88 3.56	28.7 %

\* In these cases an addition of 1.5 % of sodium chloride had been made.

TABLES II *a* AND *b*.

Changes in the serum proteins during the two stages of the heating process adopted in the method described in this paper.

In Table II *a*, during Stage I, the diluted oxalated plasma or serum, to which had been added 2 % of salt, was heated to 56°–57° for 15 hours. In Table II *b* this preliminary heating was conducted at a temperature of 58° for 7–8 hours.

In both cases the heated liquids were made 30 % of saturation with ammonium sulphate and the mixtures were then heated just to a temperature of 61° (Stage II).

TABLE II *a*. Batch T.R. 35.

	(i) Changes in the solubility of the Serum Proteins. Percentage of protein in solution calculated from the Refractometer Readings and expressed in terms of the original plasma	(ii) Changes in the precipitability of proteins by 30 % of saturation with $\text{Am}_2\text{SO}_4$ . Percentage of protein in solution in the filtrates from making the serum in (i) 30 % of saturation with $\text{Am}_2\text{SO}_4$
STAGE I. <i>The prolonged heating of the diluted serum + 2 % NaCl at 56°–57° for 15 hours.</i>		
Serum + $\frac{1}{3}$ its vol. of $\text{H}_2\text{O}$ + 2 % NaCl (=A)	6.16	5.90
Serum + $\frac{1}{3}$ its vol. of $\text{H}_2\text{O}$ + 2 % NaCl heated to 56°–57° C. for 15 hours (=B)	5.90	3.90
STAGE II. <i>The rapid heating of B made 30 % with <math>\text{Am}_2\text{SO}_4</math> just to 61° C.</i>		
Mixture B made 30 % of saturation with $\text{Am}_2\text{SO}_4$	—	3.90
do. do. heated just to 61° C.	—	3.90

Thus during the 15 hours' heating in Stage I there has been an *increased precipitation of 34.5 %* of the soluble protein by 30 % of saturation with ammonium sulphate. During the heating process in Stage II there has been no further conversion of soluble into insoluble protein.



TABLE II b. *Batch T.R. 38.*

	(i) Changes in the solubility of the Serum Proteins. Percentage of protein in solution calculated from the Refractometer Readings and expressed in terms of the original plasma	(ii) Changes in the precipitability of proteins by 30% of saturation with $\text{Am}_2\text{SO}_4$ . Percentage of protein in solution in the filtrates from making the serum in (i) 30% of saturation with $\text{Am}_2\text{SO}_4$
STAGE I. <i>The prolonged heating of the diluted serum + 1½ % NaCl at 57°–58° for 8 hours.</i>		
Serum + ½ its vol. of $\text{H}_2\text{O}$ + 1½ % NaCl (= A)	7.10	5.52
Serum + ½ its vol. of $\text{H}_2\text{O}$ + 1½ % NaCl after being heated to 57°–58° C. for 8 hours (= B)	6.99	3.76
STAGE II. <i>The rapid heating of B made 30 % with <math>\text{Am}_2\text{SO}_4</math> just to 61° C.</i>		
B made 30 % of saturation with $\text{Am}_2\text{SO}_4$	—	3.76
do. do. and heated just to 61° C.	—	3.76

During the 8 hours' heating of the serum in Stage I there has been an increased precipitation of 31.3 % of the soluble protein by 30 % of saturation with ammonium sulphate. During the heating process in Stage II there has been no further conversion of soluble into insoluble protein.

TABLE III.

A comparison between the results obtained for the concentration of antitoxic sera by the Banzhaf One Fraction method and by the method advocated in this paper.

Sera concentrated by Banzhaf's One Fraction method	Sera concentrated by the new method given above
<b>A. <i>Diphtheria Antitoxic Sera.</i></b>	
Batch No. D.R. 31 (50 litres of oxalated plasma)	Batch No. D.R. 33 (100 litres oxalated plasma)
<i>Before concentration :</i>	<i>Before concentration :</i>
Unitage per c.c. = 500	Unitage per c.c. = 275
Protein content = 7.00 %	Protein content = 7.39 %
<i>After concentration :</i>	<i>After concentration :</i>
Unitage per c.c. = 2000	Unitage per c.c. = 2200
Protein content = 20.10 %	Protein content = 17.5 %
<b>Loss of antitoxic units during the process = 12 %</b>	<b>Loss of antitoxic units during the process = 8 %</b>
<b>Amount of protein removed = 25.4 % of total</b>	<b>Amount of protein removed = 70.4 % of total</b>
<b>Potency increased by 4 times</b>	<b>Potency increased by 8 times</b>
<b>B. <i>Tetanus Antitoxic Sera.</i></b>	
Batch No. T.R. 31 (100 litres)	Batch No. 35 T.R. (100 litres)
<i>Before concentration :</i>	<i>Before concentration :</i>
Unitage per c.c. = 175	Unitage per c.c. = 100
Protein content = 6.99 %	Protein content = 6.47 %
<i>After concentration :</i>	<i>After concentration :</i>
Unitage per c.c. = 850	Unitage per c.c. = 900
Protein content = 20.2 %	Protein content = 19.0 %
<b>Loss of antitoxic units during the process = 20 %*</b>	<b>Loss of antitoxic units during the process = 10 %</b>
<b>Amount of protein removed = 40.4 % of total</b>	<b>Amount of protein removed = 68.9 % of total</b>
<b>Potency increased by 5 times (nearly)</b>	<b>Potency increased by 9 times</b>

\* In this particular concentration the percentage loss of antitoxic units was greater than usual. Our average loss by the Banzhaf One Fraction process is of the order of 10 %.

## A SUGGESTION AS TO THE CAUSE OF THE LESSENEO PRODUCTION OF INDOL IN MEDIA CONTAINING GLUCOSE.

BY ANNIE HOMER,

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*(From the Laboratories of the University of Toronto.)*

IN the *British Medical Journal*, Dec. 18, 1915, there is a letter by Mr Benians, in which he refers to the Toxic Bodies of the *Bacillus of Malignant Oedema*, and discusses the production of aromatic bodies by the action of bacteria on proteins. He also speculates as to the cause of the lessened production of indol in peptone culture media containing glucose, glycerine and other similar bodies. As the metabolic activities of some bacteria decompose these substances with the production of acid, Mr Benians wonders whether it is this acidity which so modifies the action of the proteolytic ferment that cultures, which, in the ordinary course of events are very foul remain sweet when glucose is present.

In this connection the following observations may be of interest even though, owing to unforeseen difficulties, the work as originally planned is not yet completed.

The experimental work detailed in this communication was undertaken with the object of ascertaining to what extent tryptophane is decomposed by various organisms into indolpropionic and indolacetic acids and into indol and skatol. The organisms were grown on a synthetic medium containing tryptophane and the production of the decomposition products of tryptophane was detected colorimetrically by means of the nitrite and the p. dimethyl amidobenzaldehyde tests.

In a previous communication I have shown that, in order to apply these colorimetric tests for the presence of indol, skatol, indolacetic and indolpropionic acids, it is necessary to subject the liquids containing them to a preliminary process of separation. Extraction of the solutions

with ether will remove the soluble indol, skatol, indolacetic and indolpropionic acids from the ether insoluble tryptophane. A further separation may be effected by steam distillation of the ether extract: indol and skatol being carried over in the steam whereas indolacetic and indolpropionic acids remain behind. The individuals of these pairs can be distinguished from each other as, fortunately, they give different reactions with the colorimetric reagents employed.

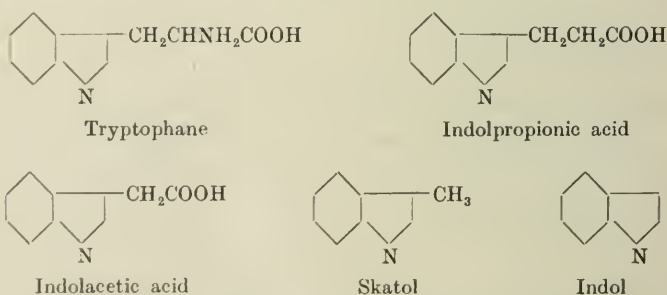
Two synthetic media were employed, the one A containing the necessary nutrient salts, 1 % of gelatine and 0.15 % of tryptophane, the other B containing in addition 1 % of glucose.

After sterilisation the tubes containing the media were inoculated with as many strains of aerobic organisms as were available and the various indol products were tested for colorimetrically.

It was noticed that where growth took place it was more luxuriant in the medium A than in the medium B: in fact in several cases while there was growth in A there was none in B.

It was also observed that the indol or indolacetic acid production by the various organisms was greater in A than in B. This phenomenon may be due to one of two causes: either that the organisms find it easier to derive their energy from the decomposition of glucose than of tryptophane, or else that the glucose by chemically reacting with tryptophane produces a substance or substances not so readily useful as is tryptophane for bacterial metabolism.

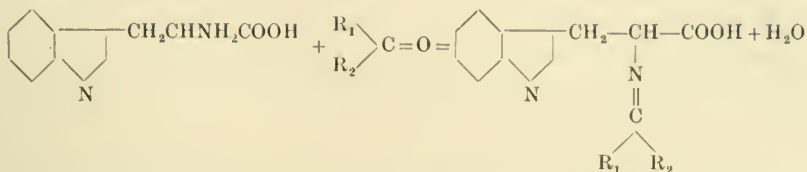
The chemical reactions involved in the formation of indol, skatol, indolpropionic and indolacetic acids from tryptophane necessitate the preliminary removal of the *amino* ( $\text{NH}_2$ -) group from the side chain of the molecule thus:



Now, it has been shown that the *amino* group of tryptophane will readily react at body temperature with the *carbonyl* group of an aldehyde or of a ketone to form colourless compounds of the type:



## Type I.



These compounds, of which several have been isolated and of which a few have been analysed (Homer, *Biochem. Journ.* 1913, VII, 101), are less readily attacked by various chemical reagents than tryptophane itself.

I therefore venture to suggest that in the medium containing glucose there is lessened indol production because of the formation of a glucose-tryptophane complex which is not so readily attacked as tryptophane alone. Moreover, in a medium in which the tryptophane is rendered chemically unsuitable for bacterial decomposition there is impaired growth of the organisms because, once the tryptophane is inactivated, gelatine alone cannot be regarded as a satisfactory substitute for the protein constituent of a culture medium. Gelatine lacks tryptophane, a substance which Hopkins and Willcock (*Journ. Physiol.* xxxv, 88) and Osborne and Mendel (*Journ. Biol. Chem.* 1914, XVII, 325) have shown to be necessary for the maintenance of the life of the individual.

It was further noticed that in the course of the sterilisation the colour of B became more intense than that of A, and further, during the incubation period the intensity of colour of both media became more marked: A becoming yellow while B became dark brown, almost black, owing to the formation of the so-called "humin-like" substances. This intense browning of the colour was a special feature of the tryptophane-glucose combination as it did not take place in the media containing tryptophane and gelatine but no glucose, nor did it occur in media containing glucose and gelatine but no tryptophane.

Now, tryptophane and other indol compounds and pyrrol derivatives by virtue of their *imino* (-NH-) group will readily react with the *carbonyl* group of aldehydo- or keto-compounds to form condensation products of intense colour. The tryptophane and other indol complexes of this type vary in colour from yellow or red to brown or black (Homer, *loc. cit.*). Their constitution has not yet been determined but the available data seem to indicate that at least two indol nuclei are involved in the condensation. Thus in the case of indol:



The compounds of this type are markedly stable.

From these considerations it is probable that the production of the "humin-like" substance characteristic of the glucose-tryptophane medium B is due to the formation of an intensely coloured compound of Type II by the chemical interaction of glucose and tryptophane or of glucose and the previously mentioned glucose-tryptophane complex of Type I.

We thus see that in a liquid culture medium containing glucose and substances such as peptone, which on hydrolysis give rise to tryptophane, there is the possibility of the formation of two types of condensation products between glucose and tryptophane. The formation of a compound of what I have designated Type I is probably responsible for the lessened production of indol and other foul smelling indol substances and the formation of substances of Type II is probably the cause of the darkening during sterilisation and incubation.

## BACTERIOLOGICAL ASPECTS OF THE MENINGOCOCCUS CARRIER PROBLEM<sup>1</sup>.

By A. EASTWOOD, M.D.

### INTRODUCTION.

DURING the year 1915, Drs F. Griffith, W. M. Scott and I have been investigating the meningococcus in the Board's Pathological Laboratory with the object of providing information which may be of service to the Board, particularly with reference to the carrier problem.

Before entering into technical details I wish to state in general terms the problems which present themselves and the difficulties which are to be overcome.

There is an obvious difference between diagnosing the meningococcus in cerebro-spinal fluid withdrawn from a patient with symptoms of meningitis and identifying this organism in a culture from the naso-pharynx of a person not clinically affected with the disease. Assuming the two organisms to be alike in cultural characters, with the former the confirmatory evidence of specific pathogenicity is supplied by the condition of the patient; with the latter organism, this confirmatory proof is lacking. In the case of such organisms as the bacilli of plague, tuberculosis, or anthrax, the proof could readily be provided by animal experiment; but for the meningococcus, as for the typhoid bacillus, there is no laboratory animal available for routine demonstration of specific pathogenicity. It is therefore necessary to be content with indirect evidence that an organism isolated from the naso-pharynx is capable of producing cerebro-spinal fever.

The question then arises whether morphological and cultural characters alone, if worked out with sufficient care, can be accepted as giving complete identification. These characters, including fermentation tests,

<sup>1</sup> Reprinted by permission of H.M. Stationery Office, from *Reports to the Local Government Board on Public Health and Medical Subjects*, n. s. No. 110 (1916). ED.

are certainly of very great aid to diagnosis, and there is practically complete agreement about what cultural characteristics are to be regarded as typical of the true meningococcus. As, however, some little differences of opinion have been expressed regarding the fermentation of sugars, this question will be treated in this report as a problem calling for consideration.

But, assuming this question of fermentation reactions to be settled, it must still be admitted that more confidence would be felt in the fermentation tests if they could be confirmed by other tests of a more specific nature. This naturally suggests an appeal to serological reactions. If, for example, strains from the naso-pharynx agreed with undoubted meningococci, not only in cultural and fermentation tests, but also in agglutination with a specific serum, their identity might be admitted; and, if the correspondence between fermentation and agglutination tests was a regular occurrence, the question might be raised whether resort to the latter might, except under exceptional circumstances, be considered unnecessary for routine diagnosis. But here a preliminary and very serious difficulty arises owing to the fact that different strains of undoubted meningococci differ among themselves in their serological reactions; and no serum is available which can be guaranteed to agglutinate every strain of meningococcus without fail. Obviously, here is a problem where research is needed.

It will probably be a long time before a complete scientific explanation is accepted for the serological reactions of all strains of meningococci isolated from cerebro-spinal fever; but, in the meantime, this consideration is no bar to comparison, with such sera as are available, between the agglutination of undoubted meningococci and that of strains isolated from the naso-pharynx. It is clearly a matter of immediate interest to see if identity can be established between the former and the latter.

Difficulties in serological work with the meningococcus, though still very considerable, are not now, and never have been, of such a nature as to render such investigations useless. Ten years ago identity in serum reactions had been established between certain strains from cerebro-spinal fluid and certain strains from the naso-pharynx. These latter strains had been mainly derived from contacts, but they had also, though in much smaller numbers, been obtained from non-contacts.

This last fact raises a question which demands urgent consideration at the present time. What practical advantage is to be gained by culturing swabs from the naso-pharynx as a routine aid to prophylactic



measures? It cannot be taken for granted, without adequate bacteriological evidence, that the meningococcus is so frequently present in the normal, or merely catarrhal, throat as the pneumococcus, or, possibly, the influenza bacillus. But still it may come under the same stigma of the bacteriological class of "common lodger," which, though more frequently innocuous, or relatively so, is capable, under favourable conditions of reduced resistance, of producing its specific disease. If that is the case, the meningococcus carrier must obviously be placed in a different class from, for example, the typhoid carrier. So the practical question is, Is it true that in this country and at the present time, when precautions have to be taken against the spread of cerebro-spinal fever, the meningococcus is to be found widely distributed in the throats of persons not known to have been in contact with the disease?

This is the main problem which has been engaging the attention of the Board's Laboratory. The investigation has involved research in the directions I have already indicated, viz.:—(1) a comparative study of the fermentation and serological reactions of various strains of meningococci derived from the cerebro-spinal fluid of patients affected with meningitis; (2) a similar study of various meningococcus-like organisms obtained from the throats of non-contacts; (3) a comparison between the cerebro-spinal strains and the throat strains.

Prior to the outbreak of the epidemic in this country towards the end of 1914, many investigators have worked on the meningococcus and have recorded their results in various scientific publications. As much of their laboratory data has an important bearing on the present enquiry, I have collected and reviewed the matter which seems to me of main importance in connection with the work done in the Board's Laboratory.

#### HISTORICAL SURVEY.

It is not necessary to revert to the earlier literature, written when the diplococcus of Weichselbaum was on its trial. I commence with the year 1906, when the causal relationship of this organism to cerebro-spinal fever was fully established, and its cultural characters had been thoroughly worked out. It will be convenient to dispose of fermentation tests before entering into the more complicated subject of serum reactions.

*Fermentation Tests.*

Lingelsheim (1906)<sup>1</sup> made a thorough investigation of the use of fermentation reactions as a means of distinguishing meningococci from other Gram-negative diplococci found in the naso-pharynx. He used solid media (ascitic agar) containing 1 % of the substance to be tested. This had previously been sterilised in 10 % solution with Kubel-Tiemann litmus and was added to the ascitic agar just before pouring the medium. He found that meningococci produced an acid reaction in the media containing dextrose and maltose, but gave no evidence of the formation of free acid in media containing laevulose, galactose, mannite, dulcite, saccharose, lactose, or inulin. These results he confirmed with 83 strains of meningococci; they all behaved in the same way, and the fermentation with dextrose and maltose was invariably well marked.

Buchanan (1907)<sup>2</sup> used for his fermentation tests a solidified medium composed of 3 parts ox-blood serum, 1 part bouillon, with 1 % of glucose, galactose, maltose, and saccharose, respectively, 1 in 10,000 of neutral red being added as indicator. He examined the throats of contacts during the Glasgow epidemic and obtained 81 positive cases (26·3 %). Cultures from each of these were tested on the sugar media and gave, in every case, an acid formation with glucose and maltose, but never with saccharose. On several occasions a slight amount of acid production was observed in the galactose tube, but, he states, "this reaction is so trivial and so seldom met with that the meningococcus may be held as giving a negative reaction with galactose in this medium." He adds that in a fluid medium, containing galactose and ascitic fluid, an acid reaction has been found after an interval of several days. He noted that in his solid glucose and maltose media the meningococcus turned the water of condensation a bright yellow colour with a greenish fluorescence and a yellow, pus-like deposit at the bottom of the tube. These features he regarded as characteristic. In his table comparing the meningococcus with other varieties of Gram-negative diplococci found in the naso-pharynx, one of the latter (No. 4) is stated to agree with the meningococcus in fermenting glucose and maltose alone and in not growing at 23°–25° C., but to differ in the following respects:—(1) on glucose and maltose tubes, "growth at first resembles meningococcus, but in 48 hours the medium becomes

<sup>1</sup> *Klin. Jahrb.* xv.

<sup>2</sup> *Trans. Internat. Congr. on Hygiene and Demography, Berlin, September, 1907, iv.*

reddened throughout"; (2) in plate culture, "colonies on Petri plate are smaller than meningococcus and become intensely red in centre."

Gordon (1907)<sup>1</sup> recommended, for the study of the fermentation reactions, a Lemco-peptone medium containing 1% of the required carbohydrate; to this, after sterilisation, a little sterile raw ascitic fluid was added before inoculation. The meningococcus, he found, was characterised by producing an acid reaction with glucose, galactose, and maltose, but not with saccharose.

Arkwright (1907)<sup>2</sup> employed liquid media for the purpose of fermentation tests and found that the characteristics of the meningococcus were "the production of acid from maltose and usually from glucose, galactose, and laevulose, but not from cane sugar."

Shennan and W. T. Ritchie (1908)<sup>3</sup> studied the fermentation reaction of 19 strains of meningococci obtained from cerebro-spinal fluid, using solid media prepared after the method of Lingelsheim. Acid production was always observed with glucose and maltose and, in five instances, with dextrin; the reactions were negative with galactose, laevulose, cane sugar, lactose, dulcitol, mannitol, inulin, and raffinose. The authors found that the alkalinity of their media should be very slight, as slight increase of alkalinity might prevent the appearance of an acid reaction. Two of their strains, though giving distinct reactions with maltose, gave comparatively slight reactions with glucose. Comparing liquid with solid media (parovarian broth with parovarian agar), they found that when galactose was added, seven of their strains produced acid in the liquid medium, but not in the solid.

Mayer (1909)<sup>4</sup> used Lingelsheim's solid media for his fermentation tests of a large number of naso-pharyngeal and cerebro-spinal strains which he investigated during the occurrence of cerebro-spinal fever at Würzburg. All his strains of undoubted meningococci agreed in fermenting glucose and maltose only; saccharose, lactose, laevulose, and galactose being negative. They produced a well marked reddening of the medium with maltose, but only a slight reddening with glucose. Three strains, not classed as meningococci, are of interest in that the reddening with glucose was well marked, while in all other cultural respects they were practically identical with meningococci. Apparently they are excluded from this class because one of them was only

<sup>1</sup> Report to the Local Government Board on the Micrococcus of Cerebro-Spinal Meningitis and its Identification.

<sup>2</sup> *Journ. of Hyg.* VII, 145.

<sup>3</sup> *Journ. of Path. and Bact.* XII, 456.

<sup>4</sup> *Centrbl. f. Bakteriolog. Orig.* XLIX.



agglutinated up to 1 : 250 with the Höchst meningococcus serum, and the other two were not agglutinated above 1 : 100; whereas the undoubted meningococci were agglutinated up to 1 : 500. A fourth strain also bore a close cultural resemblance to the meningococcus, but differed in that with maltose, as well as with glucose, the reddening of the medium was only slight. This strain gave an agglutination with the Höchst serum in 1 : 100.

When, instead of using Lingelsheim's medium (ascitic agar), he added the litmus-sugar solution to Kutscher's agar medium, which is made with broth prepared from human placenta and contains ox serum in place of ascitic fluid, he obtained the most confusing results. Testing five strains which gave what he regarded as the typical reactions of the meningococcus on Lingelsheim's media, he found that one gave no evidence of acid formation with any of the four sugars—glucose, maltose, galactose, lactose; one produced acid with maltose only; the remaining three produced slight acid with all four sugars. He thought this difference might be attributable to the presence of dissolved red blood corpuscles in the medium.

Arkwright (1909)<sup>1</sup> preferred fluid to solid media for fermentation tests, as he found that the change with the latter, though usually more rapid, was in many instances of a very transient character. Testing 36 strains in weak broth with the addition of serum and one or other of the sugars—glucose, maltose, laevulose, and saccharose, he found that 17 fermented the first three, 15 the first two only, 1 glucose and laevulose, 1 maltose only, and 2 fermented none of the sugars.

Symmers and Wilson (1909)<sup>2</sup> applied fermentation tests to strains of meningococci obtained during the Belfast epidemic, using the fluid medium recommended by Gordon. They found that glucose, maltose and dextrin always produced acid and that all the other substances employed, including galactose and laevulose, failed to do so. 53 strains were tested with galactose and 43 with laevulose.

Elser and Huntoon (1909)<sup>3</sup> went very fully into the fermentation reactions of meningococci. They used Kahlbaum's and Merck's guaranteed pure products of dextrose, galactose, laevulose, lactose, maltose, saccharose, mannite, dulcitol, inulin, and dextrin. These were sterilised in 10 % solution and then added in requisite quantity to the media. Various liquid media were tried, but though these furnished "reliable data concerning the fermentative capacities," they

<sup>1</sup> *Journ. of Hyg.* ix, 104.

<sup>2</sup> *Ibid.* p. 9.

<sup>3</sup> *Journ. of Med. Research*, xx, 377.



were not recommended, because failure to grow was common. They therefore adopted for routine use Lingelsheim's method of using solid media, with a basis of ascitic agar, but tubed their medium instead of plating it. Two hundred strains of meningococci were tested and were found to agree in fermenting dextrose and maltose only. They were aware that some observers, using liquid media, found that, in addition to these two sugars, one or other of the products, laevulose, galactose, and dextrin might also give rise to an acid reaction. Laevulose and galactose, they showed by experiment, are particularly liable to be altered by sterilisation, and they quote Maquenne, who found that so-called chemically pure dextrin contains maltose, iso-maltose, or glucose in small quantities.

With liquid media the acid reaction produced by glucose and maltose usually required 48 hours to develop, and sometimes 72 hours, but with solid media it was usually apparent within 24 hours. Some quantitative determinations of acid production with these two substances were made and it was found that "the general average acid production is greater in maltose media than in dextrose media, a fact which was also observed in connection with the qualitative tests." But with a few of their strains dextrose produced more acid than maltose.

Blair Martin (1910)<sup>1</sup> compared the fermentation reactions of the meningococcus and the gonococcus, using solid culture media. He found that all his strains of meningococci, 31 in number, agreed in producing acid with maltose and dextrose, and in failing to do so with laevulose and saccharose. He noted that some of his strains of meningococci fermented dextrose less rapidly than maltose.

#### *Conclusions as to Fermentation Tests.*

Lingelsheim deserves the credit of having shown that litmus-ascitic-agar is a thoroughly reliable medium as the basis for these tests, and that, on this medium, the meningococcus produces free acid with both glucose and maltose, but not with laevulose, galactose, mannite, dulcite, saccharose, lactose, or inulin. These results have now been corroborated on a large scale by many independent observers and must be accepted as accurate. Care must, of course, be taken in the preparation of the medium; the meningococcus is a somewhat feeble acid producer and if the medium be too alkaline the reaction may be masked.

<sup>1</sup> *Journ. of Path. and Bact.* xv, 76.

Another point to bear in mind is that occasionally, though rarely, a strain is found which does not give these reactions until it has been in subculture for some time.

As regards the comparative intensities of the reactions in the glucose and the maltose media, it is interesting to note that some observers have found the reaction equally well marked with both; others that more acid is liberated with maltose than with glucose; and others, again, that with the majority of strains maltose gives more acid than glucose, but that with some the reverse is true.

Several of the observers who have placed reliance on solid media have shown that when liquid media are used some differences may be found, the most noteworthy being the production of acid with galactose. Elser and Huntoon, in particular, have done valuable service in clearing up apparent discrepancies between the results obtained with liquid and with solid media by demonstrating that certain of the sugars, especially laevulose and galactose, are, when sterilised in liquid media, especially liable to modifications which enable the meningococcus to form acid from them.

As regards Buchanan's solid medium, in the report which I have quoted he apparently excludes certain strains from the class of genuine meningococci for the one reason that they produce with glucose and maltose a more intensely acid reaction than the genuine meningococcus. This is an interesting observation, but the exclusion obviously requires confirmation by more specific tests.

#### *Serum Reactions.*

Lingelsheim (1906)<sup>1</sup> tested the agglutinability of a large number of strains of meningococci which he had collected during the epidemic in Upper Silesia in the winter of 1904-5.

His strains were obtained partly from cerebro-spinal fluid and partly from pharyngeal swabs. He employed the macroscopic method, using 1 c.c. of fluid, in which one normal loopful of culture was emulsified, and incubated his dilutions for 20 hours at 37° C. In the course of his work he found that several circumstances affected the agglutinability of the same strain with the same serum. When a culture was emulsified and tested at once, without preliminary treatment of any kind, it showed much less agglutinability than it did when the emulsion had been kept, at room temperature, for some time. This effect of storage

<sup>1</sup> *Klin. Jahrb.* xv.

was not confined to a particular strain, but was found to be a general rule. During the first three to four weeks of storage, the agglutinability was unstable, but after the fourth week it became constant and then remained unchanged to the end of the sixth month. Compared with its agglutinability when tested in the fresh condition, the agglutinability of a culture emulsion which had been kept until it became constant was increased about five times. The emulsions were made from ascitic agar cultures with .9 % saline solution, .1 c.c. of formalin being added to 40 c.c. of saline. Lingelsheim also investigated the influence of heat on the agglutinability of fresh cultures and found that exposure for  $\frac{1}{2}$ –1 hour to temperatures of 50° C., 60° C., and 70° C. increased agglutinability but 80° C. had the reverse effect.

He prepared two rabbit sera. The first, produced with a strain of meningococci obtained by lumbar puncture, gave a titre of 1 : 400. Sixty-three strains were tested with it, the majority being cultures from the pharynx; they were all agglutinated in dilutions of from 1 : 200 to 1 : 400, whilst none were agglutinated in 1 : 10 by normal rabbit serum. The second serum, produced by another strain of meningococci obtained by lumbar puncture, gave a titre of 1 : 800 and was tested on 47 strains. These agglutinated between 1 : 400 and 1 : 800, but were not agglutinated by normal rabbit serum in 1 : 10.

With regard to the identification of Gram-negative diplococci obtained from the naso-pharynx, Lingelsheim remarked that resort to agglutination was not usually necessary, provided that careful attention was paid to cultural and fermentation tests, but he added that *flavus* No. 3 might give trouble, if only slightly pigmented, and then might have to be differentiated by agglutination<sup>1</sup>.

In a footnote, however, he appended a further qualification to this general statement. He said he was aware that from the throats of healthy persons strains were sometimes obtained which, though corresponding in all other respects with meningococci, failed to give a specific agglutination reaction. Whether such strains should be regarded as "pseudo-meningococci" must, he considered, be left for future research to determine.

Kutscher (1906)<sup>2</sup>, in view of the discovery of the meningococcus in the throats of persons in contact with cerebro-spinal fever, thought

<sup>1</sup> In a later article (1908, *Zeitschr. f. Hyg.* LIX) he said that all three varieties of *flavus* were distinguishable from meningococci by fermenting laevulose in addition to glucose and maltose.

<sup>2</sup> *Deutsche med. Wochenschr.* 1906, Nr. 27, p. 1071.



it desirable to make control investigations upon the throats of non-contacts. At Berlin, during May and June, 1905, he examined swabs from the naso-pharynx of 104 persons, about half of whom were children. They had not been in contact with the disease and were examined at a time when cerebro-spinal fever was not present in Berlin in epidemic form. All of these failed to yield cultures of the meningococcus, though at the same time the specific organism was obtained from the throats of two persons who had been in contact with sporadic cases of the disease. As these negative results were obtained at a season of the year unfavourable for the production of catarrhal conditions, there was the possibility that the bacterial flora of the naso-pharynx might be different in the winter. So he continued his investigation of non-contacts in December, 1905, and January, 1906, when Berlin had remained completely free from both epidemic and sporadic cerebro-spinal fever for a period of six months. He swabbed 56 patients suffering from slight catarrhal affections of the upper respiratory tract, and found that 52 were negative, but cultures from four were indistinguishable from the meningococcus "morphologically, culturally, and in their immunity reactions."

For his agglutination tests he employed a powerful anti-meningococcus horse serum, using the macroscopic method. The dilutions were incubated at 37° C. for 24 hours, and controls were made with normal horse serum and with saline. His four throat strains and five cerebro-spinal fluid strains obtained from the epidemic in Upper Silesia were tested, with the result that well-marked agglutination was obtained with all, the throat strains being as highly agglutinable as the strains of undoubted meningococci. As regards the significance of these agglutination tests, he remarked that, in considering results with meningococci, as with other species of cocci, agglutination in low dilution only must be taken with reserve, but agglutination in higher dilutions, such as he obtained in this case (1 : 200, 1 : 500, and sometimes 1 : 1,000), must be regarded as "a link in the chain of proof." It must also be remembered, he added, that some strains of meningococci, like some strains of staphylococci, are only agglutinated with difficulty or are almost inagglutinable.

He also tested the above throat and cerebro-spinal strains as regards absorption of agglutinin. To 10 c.c. of a 1 : 20 dilution of the serum he added three cultures (24 hours' growth) and incubated at 37° C. for one hour, the absorption being facilitated by shaking. The clear liquid obtained by centrifuging was then tested, controls being made



with unabsorbed serum, saline, and normal horse serum. He found that different strains behaved differently. When the serum was absorbed with a cerebro-spinal strain, agglutinin was almost completely lost for some cerebro-spinal and some throat strains, but remained effective, even in high dilution, for one throat and two cerebro-spinal strains. When saturation was made with a throat strain, there was again loss of agglutinin for some cerebro-spinal and throat strains and retention of it for others, but as regards the action on individual strains, the changes effected were not identical with those produced in the former experiment. So specific absorption experiments afforded no evidence that his throat strains were distinguishable from meningococci. Meningococci, he remarked, resembled the organisms of cholera, typhoid and paratyphoid, in that different strains, when tested with the same serum under identical conditions, exhibited different degrees of capacity for combination with agglutinin.

After his experiments had reached this stage, two of his throat strains were accidentally allowed to die out. With the two remaining, further investigations were conducted. Tested by Lingelsheim's recently published (1906) method of using carbohydrate media for differential diagnosis, they conformed with the author's criterion for the meningococcus. Deviation of complement experiments made with these two strains and with strains of undoubted meningococci showed that the former prevented haemolysis in as high a degree as the latter. Rabbit sera prepared with the two throat strains agglutinated a cerebro-spinal strain of meningococci, but he had not had sufficient time, when this report was issued, to prepare very strong sera.

His conclusion was that the identity of his throat strains with the meningococcus had been definitely established in the case of the last two, and had been brought to a high degree of probability in the case of the two which died out before the work was concluded.

Hübener and Kutscher (1907)<sup>1</sup> following up Kutscher's work on the "normal carrier," examined 400 soldiers in a regiment which was free from cerebro-spinal fever, and in the throats of eight found cocci which were identical with the meningococcus in every bacteriological respect, including immunity reactions. The eight men had not been in individual contact with each other. Several months previously their regiment had been quartered, elsewhere, with a regiment in which cerebro-spinal fever had occurred.

<sup>1</sup> *Deutsche militärärztl. Ztschr.* 1907, No. 15. Quoted in *Centralbl. f. Bakteriöl.* Ref. XLII.

Eberle (1908)<sup>1</sup> tested the agglutinability of 18 strains of meningococci, all of which had been obtained from cerebro-spinal fluid during life. He used five sera, viz., the Berne, Berlin, and Merck horse sera, and two monovalent rabbit sera. He adopted the macroscopic method and made his suspensions with stored emulsions to which phenol had been added. After making parallel experiments at room temperature, 37° C., and 56° C., he gave up the first, because he found that the reactions were slower, more uncertain, and did not attain to such high dilutions as with the higher temperatures. Investigating the time requisite for the reactions, he found that two hours was useless, and that incubation overnight was necessary; eventually he decided to take his readings at the end of two days, as he noted that 24 hours was not long enough; the maximum agglutination required 38–42 hours to develop.

Marked differences were found between the agglutinability of different strains when tested with the same serum, some strains reaching up to 1 : 1000 and others only up to 1 : 20 or 1 : 50. It was also noted that individual strains did not behave uniformly towards different sera. His 18 strains could roughly be divided into good, rather poor, and poor agglutinators. The last, which for the most part had been recently recovered from patients, did not agglutinate with any of the sera higher than 1 : 50. Good agglutinators would attain reactions in dilutions varying from 1 : 1000 with one serum to 1 : 100 with other sera. When the tests with the same strain and serum were repeated at different times a general conformity of results was observed. The monovalent rabbit sera did not show a distinctive difference in agglutinability as between the homologous and the heterologous strains; the more readily agglutinated strains were agglutinated as highly as, or even more highly than, the homologous strain.

Eberle concludes by saying that the identification of meningococci from the throat is difficult, and not made easier by agglutination tests.

Arkwright (1909)<sup>2</sup> studied the serum reactions of several strains of meningococci obtained from cases of epidemic and sporadic meningitis. In his report he designates his epidemic strains by the letter E and his sporadic strains by the letter L.

All his agglutination experiments were made by the microscopic method, at room temperature, and the observations were completed at the end of two hours.

He has recorded his tests of 25 strains with serum I., obtained from a horse which, for a period of 12 months, had received repeated

<sup>1</sup> *Arch. f. Hyg.* LXIV.

<sup>2</sup> *Journ. of Hyg.* IX, 104.

injections, partly subcutaneous and partly intravenous, of the strain L 1. This strain was completely agglutinated up to 1 : 100 and partially up to 1 : 500, whilst normal horse serum gave no agglutination even in 1 : 5. Strains L 7 and L 12 were also completely agglutinated up to 1 : 100; L 7 was slightly agglutinated in 1 : 500 and L 12 was partially agglutinated in 1 : 500 and slightly in 1 : 2000. Of the remaining 22 strains, one, L 5, gave slight agglutination in 1 : 100 and also in 1 : 500; none of the others gave any agglutination in 1 : 100.

Eleven of the above 25 strains were tested with serum II. This was obtained from the same horse when, after the withdrawal of serum I., the animal had received for 10 months injections of E 12, a strain which yielded no more than partial agglutination in 1 : 5 to serum I. The tabulated results show that serum II. agglutinated L 1 slightly better than the former serum had done; but with its second homologous strain, E 12, there was no more than slight agglutination in 1 : 25. Of the remaining nine strains tested, L 7 and L 12 behaved like L 1; L 5 was now agglutinated in as high dilution as these; and another strain, E 4, now became agglutinated completely in 1 : 100 and slightly in higher dilutions. None of the rest gave as much as complete agglutination in 1 : 25.

At a later date, but without having received fresh treatment, the horse was bled again, yielding serum III. This serum differed from serum II. in that it agglutinated (complete up to 1 : 50) the homologous strain E 12. It also agglutinated, in equal degree, E 19, a strain negative to serum I., but not recorded as tested with serum II. In other respects the behaviour of serum III. towards the 12 strains tested showed only slight differences from sera I. and II.

Arkwright also mentioned briefly that he tested 22 of his strains with Kolle's anti-meningococcus serum and obtained agglutination with six in dilutions as high as 1 : 100 or 1 : 200.

In a series of absorption experiments serum I. was tested with four strains which it agglutinated relatively well, viz., L 1, L 5, L 7, L 12, and with two which were not themselves agglutinated, viz., E 5 and E 7. It was found that L 1 and L 5 absorbed agglutinin for themselves and for each other, but only slightly absorbed agglutinin for L 7 and L 12. L 7 and L 12 absorbed to a certain extent their own and each other's agglutinin, but hardly any for L 1 and L 5. E 5 and E 7 absorbed no agglutinin for any of these four strains.

These results may be compared with fixation of complement experiments with serum I. Out of the four strains mentioned above,



which agglutinated relatively well, fixation was complete or nearly complete with L 1, L 7, and L 12; with the fourth, L 5, it sometimes occurred and sometimes failed. Out of six strains which were recorded as only slightly agglutinable (1 : 5 or 1 : 20), one brought about complete, or nearly complete, deviation; the others failed.

Incidentally, on comparing Arkwright's tables, there is evidence that when the same strain was tested with serum I. on different occasions the results did not always correspond precisely. For example, out of seven tests with L 12, the result on two occasions was complete agglutination up to 1 : 200 and slight up to 1 : 500; but on another occasion the agglutination was only slight in 1 : 100 and only partial in 1 : 20 and 1 : 50.

In his "conclusions" Arkwright states that "the variations observed in the sugar and serum reactions were not such as to indicate a specific difference between the epidemic and sporadic strains, for the differences between individual members of each group were as great as any found between the two groups."

Elser and Huntton (1909)<sup>1</sup> worked for the commission appointed by the Health Board of New York in March, 1905, to investigate cerebro-spinal meningitis. After presenting an epitome of their work in January, 1906, they proceeded to further study of serum reactions and issued their final report in 1909. The majority of the strains of meningococci which they examined were obtained from cerebro-spinal fluid or meningeal exudate; they also report on other cocci of interest, which were isolated from the respiratory passages in cases of meningitis, or from persons who had come in more or less close contact with such cases.

They prepared agglutinating sera by inoculating rabbits intravenously with weekly doses of meningococci suspended in saline solution and killed by heating at 65° C. for 30 minutes. The initial dose was usually .002 gm.; this was gradually increased to .008 gm. They relied entirely on the macroscopic method of examination, as preliminary tests with the microscopic method had been found less satisfactory. Their dilutions received, per c.c., .004 gm. of moist cocci, measured with a standard platinum loop. The dilutions were incubated for two hours and then kept in the cold (9° C.) for 22 hours. The final reading was taken at 24 hours; preliminary readings were also usually taken at 1, 2, 3, and 4 hours. Occasionally, no appreciable change was noticed after several hours, although complete clarification was found in 24 hours.

<sup>1</sup> *Journ. of Med. Research*, xx.



When the agglutination was not progressive with increase of time, the tests were repeated. They recorded a reaction as positive when the great majority of the cocci were clumped and deposited at the bottom of the tube, whilst the supernatant fluid presented a slight degree of turbidity when viewed obliquely in a good light. Control tests were made with normal rabbit serum. The cultures used for testing agglutinability were grown on media which did not contain serum or ascitic fluid.

In discussing their reasons for adopting the above technique, they stated that they had made a comparative study of the influence of incubator, room, and ice-box temperature on agglutination and found that, though incubator temperature hastened the reactions, the end results were not markedly influenced. They did not agree with Kutscher that agglutinability was brought to a higher level by exposure to 55° C., though it might be accelerated. In support of these statements they have published two tables. Table X. shows agglutination tests with seven strains of meningococci at both 37° C. and 55° C., readings being taken at 1, 2, 4, and 24 hours. It is noteworthy that three of the strains were practically unaffected by the serum employed and that each of the remaining four showed more agglutination at 24 hours than at 2 hours, when incubated at 37° C. Table XI. records agglutination tests with nine strains of meningococci at temperatures of 10° C., 30° C., and 37° C., the readings being taken at 24 hours. Three of the strains were practically unaffected by the serum employed; of the remaining six, three showed most agglutination at 37° C., the remaining three were equally agglutinated at all three temperatures.

They considered it necessary to use normal rabbit serum as a control, as they found that some strains of meningococci were agglutinated by the normal serum in 1 : 50 and, rarely, in 1 : 250.

In their experience, growth on media containing serum or ascitic fluid had an unfavourable influence on agglutinability; so they waited until their cultures could be accustomed to grow without the aid of these ingredients before testing for agglutination, glucose agar being generally adopted as the most suitable medium.

They stated that "one of the great difficulties encountered in connection with this work is the very pronounced instability of the agglutinable properties of the meningococcus." When the same strains were tested on different occasions with the same sera, the results were not found to be constant. Lingelsheim, they remark, suggested that killed cultures gave better results, and in this view they agreed with him;

but they have not stated to what extent or with what degree of success they followed Lingelsheim's technique. Apparently they were unable to overcome the difficulty completely, as they have again stated, in the summary of their results, that "the unusual instability of the agglutinable properties of the meningococcus" was a serious obstacle. They tried to increase agglutinability by growing their strains in glucose broth, and reported that "while several strains reacted promptly to this treatment, others were not materially altered, even after prolonged growth, and in a few there was reduction of agglutinability."

Apart from variations in the agglutinability of individual strains, they found great differences in the degree of agglutinability exhibited by different strains in the presence of the same immune serum. They tested 65 strains with a serum, prepared from their strain M 30, which gave a titre of 1 : 1000. Twenty-five of these strains either failed to react at all or gave no more than incomplete reactions in 1 : 50. Tests have also been recorded of sera prepared from certain other strains of meningococci, and with these sera again the results showed that some strains were not agglutinated or were only agglutinated to a slight degree. Enquiring further into some of the strains which failed to agglutinate, they have reported that injection of them into rabbits produced sera which were ineffective towards the homologous strains, but were of moderate potency in agglutinating strains already found agglutinable with the sera previously mentioned. Hence they have termed the strains in question "agglutinogenic" but "inagglutinable." They admitted that this latter term was not absolutely accurate, because, though some of the strains failed to agglutinate even in 1 : 10, others were agglutinable up to 1 : 50; but they found it convenient to define as "inagglutinable" all strains which failed to react to powerful sera in 1 : 100. They remarked that they were unable to render an inagglutinable strain more sensitive by conducting the experiment at a temperature of 55° C.

Proceeding to absorption experiments, Elser and Huntoon called special attention to their technique. "Instead of exhausting a serum with large and variable quantities of bacteria, each serum was absorbed with small and constant amounts in the hope of detecting slight differences in the absorptive capacities of different strains belonging to the same species." The suspension of cocci was .004 gm. per c.c., and was mixed with equal parts of diluted serum. Two control tubes received the same amount of diluted serum and equal parts of saline. The tubes

were incubated for 2 hours. Preliminary tests showed that the maximum absorption was attained within this time; no more was obtained in 24 hours. Their attention was called to the condition of the contents of the tubes after centrifuging. The agglutinable homologous cultures were firmly packed at the bottom; the fluid was perfectly clear, and vigorous shaking failed to re-establish a perfect suspension. But the heterologous strains and the inagglutinable homologous strains were less firmly packed; the supernatant fluid was not quite clear; and vigorous shaking produced a perfect suspension.

They found that the inagglutinable strains were capable of absorption, to a certain extent, and, in general, that the binding capacity of these corresponded with their agglutinogenic capacity. There was, however, one exception. A certain inagglutinable strain was found to have no power of absorption with the serum produced from it, though this serum was capable of agglutinating one strain up to 1:1000. No definite relationship could be established between absorptive capacities and agglutinability. They have quoted, for example, an instance where an inagglutinable strain absorbed as much as an agglutinable one.

In summary of the above, a large number of strains, though undoubtedly meningococci, were more or less inagglutinable by the sera with which they were tested. The authors have made no definite statement as to the number of these strains which produced sera capable of agglutinating other meningococci but incapable of agglutinating the homologous strain.

They have recorded, however, an experiment with five strains—two “agglutinable” and three “inagglutinable.” With regard to two of the three latter, they have noted that they “were inagglutinable when first selected for the present purpose, but subsequently reacted to certain immune sera in dilutions of 1:100.” With each of the five strains one rabbit was immunised. The sera from the five rabbits were tested against 18 strains, the result being that all five sera “affected agglutinable meningococcus strains in fairly high dilutions.” Taking the results which did not attain to a positive reaction in 1:100—with the serum prepared from one of the agglutinable strains, only two of the 18 strains failed to reach this standard; with the serum prepared from the second agglutinable strain, 11 strains failed to reach the same standard; with the sera produced by the three “inagglutinable” strains, the numbers of strains which fell short of this standard were, respectively, 8, 11, and 12.

In this same experiment with the sera of five rabbits, each immunised



with a different strain, the maximum reactions of the three "inagglutinable" strains towards these five sera were:

Cultures	Sera prepared with the				
	Agglutinable strains		Inagglutinable strains		
	M 24	M 83	M 101	M 175	M 61
M 101	0	1 : 100	1 : 25	0	0
M 175	1 : 25	1 : 100	1 : 25	1 : 100	1 : 50
M 61	0	1 : 50	0	0	0

The above are all the experimental data which I can find in support of the generalised statement as regards the "agglutinogenic" capacities of their "inagglutinable" strains, which the authors make in their "conclusions." This statement is that "towards the homologous inagglutinable strains they were ineffective."

With regard to their Gram-negative diplococci, other than those regarded by them as meningococci, they found that all except six strains could be differentiated from the meningococcus by means of cultural and fermentation tests. These six, which they term "pseudo-meningococci," agreed with the meningococcus in their cultural and fermentation tests, but differed in their serum reactions. They were not agglutinated with a meningococcus serum, and were found incapable of abstracting specific agglutinin from it. They found their pseudo-meningococci more toxic for rabbits than meningococci; many succumbed to very small doses. They succeeded, however, in producing a serum (titre 1 : 500) with one strain. This serum did not agglutinate any of the other five strains of pseudo-meningococci; nor did it agglutinate strains of meningococci in higher dilution than did normal serum. Normal rabbit serum agglutinated the strain used for producing the immune serum up to 1 : 50, and a strain of meningococci (M 250) up to 1 : 100. In a series of absorption experiments with its own strain this "pseudo-meningococcus" serum lost the agglutinin to its homologous strain, but not to M 250.

Mayer (1909)<sup>1</sup> tested, with the Höchst serum, the agglutinating properties of various strains, including undoubted meningococci and organisms, obtained from the naso-pharynx, which presented certain resemblances to these. No statement is made as to how this serum was prepared. Mayer used both the macroscopic and the microscopic methods of examination. His dilutions were kept for 12 hours at 37.5° C. before the final reading. He attached particular importance to exposure to this temperature for at least 12 hours, and has recorded

<sup>1</sup> *Centralbl. f. Bacteriol. Orig.* XLIX.



experiments showing that, when the readings were taken successively at 1, 2, 3, 6, and 12 hours, standard strains did not give definite agglutination in high dilution (1 : 500) before the 12 hours' period was reached.

His standard strains of undoubted meningococci were four in number. One was obtained from Ruppel and one from Weichselbaum; the other two, designated M. 1 and W. 1, were cultured by Mayer from lumbar puncture fluid. These four all gave definite agglutination with the Höchst serum up to 1 : 500; and they were all identical morphologically, in their mode of growth with various media, and in fermentation tests.

With these four strains he compared his throat strains, which he had collected in the course of an examination of 251 persons.

Of these throat strains, thirteen, all derived from the throats of infected persons or carriers, agreed in every respect with his standard strains. Fifteen other throat strains corresponded with his standard strains in all respects except agglutination; with his Höchst serum they were either not definitely agglutinated in any dilution or at least not in a dilution as high as 1 : 500. These strains he has called "pseudo-meningococci." The remaining throat strains could be excluded from the class of meningococci without resort to agglutination tests.

He stated that amongst normal individuals, who had not been in contact with cerebro-spinal fever and were examined at a time when this disease was not prevalent, cocci might be found in the throat which could only be distinguished from meningococci by the agglutination test. He maintained that such strains could not be regarded as meningococci unless they gave a positive reaction up to 1 : 500.

Friese and Müller (1909)<sup>1</sup> found cocci closely resembling meningococci in the throats of persons who had not been associated with cases of cerebro-spinal fever. They were led to this discovery whilst investigating, at Beuthen, an outbreak amongst the troops which was regarded as influenza. These cocci were found in 28 out of 36 soldiers affected with this epidemic. Then 60 normal soldiers were taken as controls, and similar cocci were found in the throats of 28 of these. In some cases the cocci were abundant, in others only moderately numerous, and, in others, scanty. By microscopic methods and cultural tests, including fermentation tests, they were unable to distinguish them from meningococci. They therefore resorted to agglutination tests and, on the strength of the results obtained by this method, designated these cocci as pseudo-meningococci or "S. cocci."

In their agglutination work they employed the macroscopic methods

<sup>1</sup> *Klin. Jahrb.* xx.

and used 48 hours' cultures grown on slightly alkaline ascitic agar. They used cultures of this age because Lingelsheim had observed that such cultures agglutinated better than 24 hours' growths. The serum used was Merck's polyvalent horse serum, giving a titre of 1:600 with good agglutinating strains of undoubted meningococci. Their dilutions were treated in one of two ways:—(1) incubation at 37° C., preliminary reading at 24 hours, final reading at 48 hours; (2) incubation at 55° C., final reading at 24 hours.

They found that the latter method gave sharper distinctions between their "S. cocci" and undoubted meningococci. Out of 21 "S." strains tested at 55° C., only one gave a complete reaction in 1:100; whilst, out of 16 strains of true meningococci, 12 gave a complete and 4 a partial reaction in 1:200.

They also made comparative tests with a rabbit serum prepared from "S. cocci." This had been prepared with a single strain, and the fact that it was not polyvalent they found to be a disadvantage. With this serum, again, a temperature of 55° C. brought out sharper differences than 37° C. Twenty-three "S." strains were tested at 55° C.; six gave complete reactions up to 1:400, and with four others the reaction was complete up to 1:200. On making parallel tests with 13 strains of true meningococci it was found that none gave complete reactions in 1:200 and only three were complete in 1:100.

The above data illustrate the point on which Friese and Müller have laid chief emphasis, viz., the differences between their "S. cocci" and the strains of undoubted meningococci with which these were compared. At the same time they admitted that there was a relationship between "S. cocci" and true meningococci, and they considered that this relationship was shown more strongly by some strains than by others. They also found that the agglutination reactions both of their "S. cocci" and their true meningococci were liable to some degree of variation.

The following statements in their report require mention, as they have a bearing on the question whether the authors' distinction between "S. cocci" and true meningococci can be accepted without qualification.

(1) They have reported that their earliest strains of "S. cocci" agglutinated better than all the later ones and at first seemed undoubted meningococci. But unfortunately these strains died out, and as they could not be included in their comparative tests, the data as regards their agglutinability could not be ascertained.

(2) A large number of their strains, both "S. cocci" and undoubted meningococci, were tested on two or three different occasions against

the same serum. Frequently the results obtained on different occasions with the same strain did not correspond closely; and sometimes the difference was great.

For example, on June 1st, an "S." strain gave a completely negative result with Merck's anti-meningococcus serum; but on July 10th, though the control test with normal serum was again completely negative, a complete agglutination up to 1 : 100 was obtained, and there was a slight reaction in 1 : 200. These variations in agglutinability they were unable to explain. They found them to be greater with "*S. cocci*" than with undoubted meningococci.

(3) Several of the "S." strains which, at 37° C., gave complete agglutination in 1 : 100 with Merck's anti-meningococcus serum, also gave some agglutination with normal horse serum.

(4) Although the majority of their meningococci agglutinated well with the Merck serum, a few did not.

(5) In the course of their endeavours to prepare an "S." serum in rabbits, they found that, though the homologous strain was only agglutinated to a slight degree, several strains of meningococci were agglutinated more strongly.

(6) Reference has already been made to agglutination tests at 55° C. with a rabbit serum prepared from a strain of "*S. cocci*." This serum was also tested at 37° C. It agglutinated the homologous strain completely up to 1 : 800. But, out of 19 other "S." strains which were tested, only one gave complete agglutination up to 1 : 200; whereas, in parallel tests with 11 strains of undoubted meningococci, three gave complete reactions in 1 : 200. Of these three, two agglutinated well with Merck's anti-meningococcus serum, but one was completely negative.

(7) Absorption experiments at 37° C. (48 hours' reading) with three "S." strains and three strains of meningococci. Merck's serum, after saturation with one of the latter strains, lost all agglutinin, beyond 1 : 50, for these three strains; but when the serum was saturated with an "S." strain the same three strains of meningococci gave complete reactions up to 1 : 200 and partial up to 1 : 400. An "S." serum, after saturation with a strain of meningococci, gave only slight agglutination with the meningococci and the "S." strains; when the serum had been saturated with an "S." strain, it gave rather more agglutination with strains of meningococci, but none with "S." strains.

(8) Absorption experiments at 55° C. (24 hours' reading) with five strains of meningococci and five strains of "*S. cocci*." Saturation of



"S." serum with meningococci removed agglutinin for meningococci, but not for "S." strains; whilst saturation of the serum with "S." cocci removed agglutinin for both types.

Lieberknecht (1909)<sup>1</sup> examined at Posen the throats of 150 healthy school children, not known to have been in contact with cases of meningitis, and found meningococcus-like colonies in cultures from 8 per cent. In culture tests, including fermentation tests, for which he used Lingelsheim's media, they behaved like meningococci. He then proceeded to examine the serum reactions of these cocci, and to compare them with the reactions of undoubted meningococci. He used cultures not over 24 hours old, and adopted the macroscopic method. He incubated one sample of each dilution at 37° C., and a second at 55° C. In each case he took the final readings at 24 hours.

Thirteen throat strains were tested with a Berlin meningococcus serum (titre 1 : 1000). Except with two strains, the reactions, when positive at all, were better at 55° C. than at 37° C. Taking the reactions at 55° C. only:—In 1 : 100, 6 strains were negative, 3 gave slight, 2 partial, and 2 complete reactions; at 1 : 200, 8 were negative, 3 gave slight, 1 a partial, and 1 a complete reaction. None of the strains were agglutinated by normal rabbit serum in 1 : 10.

He made similar tests with the same serum applied to 12 strains of undoubted meningococci. Here again, with slight exceptions, the reactions were rather better at 55° C. than at 37° C. Taking the reactions at 55° C. only:—In 1 : 100, 1 strain was negative, 2 gave slight, 1 a partial, and 8 complete reactions; at 1 : 200, a second strain also was negative, 4 gave slight, 3 partial, and 3 complete reactions. These last 3 also gave complete reactions in 1 : 400.

Lieberknecht prepared a rabbit serum with one of his throat cultures, giving repeated subcutaneous doses of living cocci in increasing amount. He has tabulated the effects of this serum on the homologous strain and 10 other throat strains at 37° C. (reading at 24 hours). The homologous strain was completely agglutinated in 1 : 200, and partially or slightly up to 1 : 600. Two other strains behaved similarly; the 8 remaining strains were negative in 1 : 100.

He tested the same serum with 10 strains of undoubted meningococci, and has tabulated, in this case, the 24 hours' readings at both 37° C. and 55° C. Six strains, he noted, were agglutinated better at 55° C. than at 37° C. Taking only the reactions recorded as complete:—At 1 : 600, one strain was complete at 55° C., but not at 37° C.; at 1 : 400,

<sup>1</sup> *Arch. f. Hyg.* LXVIII.



the same strain was complete at both temperatures; at 1:200, a second strain was complete at 55° C. but not at 37° C., and a third strain was complete at 37° C. but not at 55° C.; at 1:100, this third strain was again complete at 37° C., but not at 55° C., the second strain was complete at both temperatures, a fourth and a fifth strain were complete at 55° C., but not at 37° C. Taking evidence of agglutination, whether complete or incomplete, 1 strain showed evidence up to 1:100, 3 up to 1:200, 2 up to 1:400, and 2 up to 1:600.

Lieberknecht has also made brief mention of some absorption experiments. Taking a specific meningococcus serum and saturating this for one hour at 37° C., with one loopful of culture to 1 c.c. of 1:20 dilution of serum, he found that a genuine meningococcus culture removed specific agglutinin, but all his throat cultures failed to do so. He has called particular attention to the fact that the throat strain which produced the rabbit serum, mentioned above, did not absorb the agglutinin for meningococci present in his meningococcus serum.

Dopter (1909)<sup>1</sup> found certain organisms which he called "para-meningococci" in the naso-pharynx of persons who had been in contact with cases of cerebro-spinal fever. Though agreeing with true meningococci in ordinary cultural and fermentation tests, he excluded them from this group owing to certain differences brought out by serological tests. They did not give specific agglutination with a meningococcus serum and failed to respond to the absorption test for specific precipitins. They agreed with meningococci, however, in fixation of complement tests. Though calling them "para-meningococci," he considered them to be nearly allied to meningococci, and advised that, from the practical point of view, persons in whose throats they were found should be treated as carriers of the meningococcus.

Mayer, Waldmann, Fürst and Gruber (1910)<sup>2</sup> have stated that, at a time when cases of cerebro-spinal fever were found, they examined, in various garrisons, the throats of 1911 healthy persons, and found 47 (2.46 %) to be carriers. None of these developed the disease. They controlled these results by examining garrisons at Munich in 1910, when there was no cerebro-spinal fever. They swabbed the throats of 9111 men, and found 158 carriers (1.73 %). None of these developed cerebro-spinal fever.

They concluded that the meningococcus must be regarded, for practical purposes, as ubiquitous, and that bacteriological examination

<sup>1</sup> *C. R. Soc. de Biol.*, July, 1909.

<sup>2</sup> *München. med. Wochenschr.*, July 26th, 1910.

of throats was impracticable as a useful aid to prophylaxis in time of epidemic.

Sachs-Miike (1911)<sup>1</sup> enquired at Beuthen, in the winter months of the year, into the occurrence of meningococci and pseudo-meningococci in the naso-pharynx of normal persons. Out of 202 soldiers, none of whom were associated with cerebro-spinal fever, he found no carriers of true meningococci, but 15 carriers of pseudo-meningococci or "S." cocci. There was no evidence that these carriers had infected each other, as they were widely distributed in different quarters. The "S." coccus was obtained also from the throat of 1 out of 28 civilians, not associated with cerebro-spinal infection. He also took the opportunity, at the same time of the year, of examining the throats of a batch of recruits newly arrived from other parts of the country. In none of these did he find either genuine meningococci or "S." cocci.

The "S." cocci fermented dextrose and maltose, but never laevulose; and in the other routine cultural tests they were indistinguishable from true meningococci, though perhaps the outline of the primary colonies was less sharply circular, and there were fewer tetrads. Differentiation was effected by serological reactions, upon which he proceeded to report.

His agglutination tests may be summarised as follows:—

I. (a) Agglutination tests at 37° C.; reading at 48 hours. Sixteen "S." strains tested with Merck's polyvalent meningococcus serum (titre 1 : 600). The total number of tests was 35, many strains being tested twice or oftener. The results of different tests with the same strain did not always coincide precisely. In all the 35 tests some reaction was obtained in 1 : 100, the controls being all negative, and in all except 6 tests the reaction was complete in 1 : 100. At 1 : 200, only six of the 35 tests were negative; and, in this dilution, six strains were completely positive on at least one occasion. At 1 : 400 no test gave more than a partial reaction; and, in this dilution, 14 of the tests were completely negative.

I. (b) Agglutination tests at 55° C.; reading at 48 hours. The same strains and the same serum as in I (a). At 1 : 100, none of the tests was completely positive, but 11 out of the total 36 tests gave a partial reaction. At 1 : 200 all the tests were negative.

II. (a) Agglutination tests at 37° C.; reading at 48 hours. Twenty strains of meningococci tested with Merck's polyvalent meningococcus serum. The total number of tests was 48. At 1 : 100, only one test

<sup>1</sup> *Klin. Jahrb.* xxiv, 225, 451.

was negative; this was with strain 13; this strain was tested twice; on the second occasion it gave a complete reaction in 1:100. At 1:200 all the tests were completely positive, except 3, viz., the two tests with strain 13, which were completely negative, and one of the two tests with another strain, which gave a partial reaction. At 1:400 there were 10 completely negative and two partial results, all the remaining tests being completely positive.

II. (b) Agglutination tests at 55° C.; reading at 24 hours. The same strains and the same serum as in II (a). Strain 13 was negative as before (three tests). All the other tests gave completely positive reactions in 1:400.

III. (a) Agglutination tests at 37° C.; reading at 48 hours. Seventeen "S." strains tested with the Beuthen Institute's polyvalent "S." serum. Total number of tests, 30. At 1:100 every test gave some reaction, and all except four were complete; each strain gave at least one complete reaction. At 1:200 every test gave some reaction, but six were incomplete, viz., the four which were incomplete at 1:100 and two others. At 1:400, 16 tests were completely positive, five completely negative, and nine partial. Reviewing the tests as a whole, there was no consistently weak agglutination, but some little irregularity.

III. (b) Agglutination tests at 55° C.; reading at 24 hours. The same strains and the same serum as in III (a). At 1:100 five of the 17 strains failed completely; each of the remaining 12 gave at least one completely positive reaction. At 1:200 only six strains gave, each on one occasion, a completely positive reaction. At 1:400 there were only four completely positive reactions; and at 1:600 there was only one.

IV. (a) Agglutination tests at 37° C.; reading at 48 hours. Twenty strains of meningococci tested with the Institute's polyvalent "S." serum. Each strain tested twice, except one, which was tested once, and one which was tested three times. Strain 13 (see previous tests) was completely blank in both tests. The strain which was only tested once was also completely blank. One strain was completely blank in one test, but positive up to 1:100 in the second test. One strain, also completely negative in one test, was positive up to 1:400 in the second test. In summary:—At 1:200 there were 28 completely positive results, belonging to 15 strains; at 1:400 there were 17 completely positive results, belonging to 11 strains.

IV. (b) Agglutination tests at 55° C.; reading at 24 hours. The same strains and the same serum as in IV (a). Strain 13 was again

completely blank in both tests. Eight other strains were completely blank in one test, but gave some agglutination in a second; in one of these instances the agglutination was complete up to 1:400. In summary:—At 1:200 there were 22 completely positive results, belonging to 13 strains; at 1:400 there were 10 completely positive results, belonging to six strains.

Sachs-Mücke also made some absorption experiments, using Merck's polyvalent meningococcus serum (titre 1:600) and the Beuthen Institute polyvalent "S." serum (titre 1:400) against eight strains of meningococci and seven of "S." cocci. He added a 48 hours' ascitic agar culture to 5 c.c. of a 1:20 serum dilution. His tables show the following results:—

I. (a) Meningococcus serum saturated with meningococci. "S." cocci showed some incomplete agglutination at 37° C., but were completely negative at 55° C.

I. (b) The same serum saturated with "S." cocci. Meningococci agglutinated.

II. (a) "S." coccus serum saturated with "S." cocci. Meningococci agglutinated.

II. (b) The same serum saturated with meningococci. Agglutinins for "S." cocci not affected at 37° C., but completely removed at 55° C.

Arkwright (1911)<sup>1</sup> recorded further work on serum reactions, which he employed to compare the meningococcus with the gonococcus by the complement fixation test. In so far as the comparative reactions of different strains of meningococci are concerned, the following points in his article may be quoted.

Referring to his previous work on agglutination, he states that, "when working with a monovalent meningococcal serum, the number of strains agglutinated was very limited, and, even when a polyvalent serum of a titre of 1:1000 made by injecting twelve different strains of meningococcus was employed, strains of meningococcus were easily found which were not agglutinated more highly by the specific serum than by normal serum."

In his fixation experiments he used extracts of meningococci. An emulsion of the growth on ascitic agar was made with 10 c.c. of saline. "This was centrifuged and the deposit made up to its original volume with salt solution. After adding a few drops of chloroform and shaking, the emulsion was left at room temperature for three or four days. The extract was then centrifuged before use. It was

<sup>1</sup> *Journ. of Hyg.* xi, 515.



found that if the deposit from the last centrifuging was again made up to the original volume with salt solution and left for a further period of two to three days, a second extract as good as the first could be obtained, and by again repeating the same process, a third and even a fourth extract could sometimes be obtained of almost undiminished value for complement fixation experiments."

The following is a summary of his experiments with meningococcal sera and extracts:—

(1) A monovalent serum was prepared from a horse by inoculation with M 12, a culture isolated from the meninges of a sporadic case of meningitis. This serum was tested with three strains, M 119, isolated from the spinal cord of a case of acute epidemic meningitis, and M 141 and M 162, each isolated from cerebro-spinal fluid of sporadic cases of meningitis. A positive complement fixation reaction was obtained with M 141; M 119 was negative; M 162 was negative on one occasion, and in a second test it gave only a very slight reaction.

(2) A polyvalent serum was prepared from a horse by inoculation with 20 strains of meningococci. This serum was tested with the above three strains. It was positive with M 141 and M 162, but negative with M 119.

(3) A monovalent serum was prepared from a rabbit by inoculation with M 141. This was tested with the homologous strain and also with M 162, M 135, M 164, and M 165. M 135 and M 164 were isolated from cerebro-spinal fluid of sporadic cases of meningitis; M 165 was isolated from cerebro-spinal fluid of a case of meningitis occurring in an epidemic area. On one occasion the serum gave positive reactions with all five strains; on a second occasion the reactions with M 162 and M 164 were very slight.

(4) A monovalent serum was prepared from a rabbit by inoculation with M 162. This was tested with the homologous strain and also with M 135 and M 164. It gave positive reactions with all three.

Dopter (1911)<sup>1</sup> reported the discovery of his "para-meningococcus" in seven sporadic cases of cerebro-spinal fever. These cases were all typical, clinically, and all ended fatally.

Dopter (1912)<sup>2</sup> reported that, up to date, he had collected 12 cases of para-meningococcal cerebro-spinal fever. Clinically the cases were typical of cerebro-spinal fever, but the organisms were not agglutinated with anti-meningococcus serum, and such serum was of no therapeutic

<sup>1</sup> *Bull. Soc. Méd. des Hôp. de Paris*, xxxi, 590.

<sup>2</sup> *Ibid.* June 14th, 1912, p. 828.

benefit. Success in treatment was, however, attained in cases where anti-para-meningococcal serum was employed.

Darré and Dumas (13, vi. 1914)<sup>1</sup> reported on cultures which they obtained from two cases of cerebro-spinal fever, one an adult and the other a child. In cultural and fermentation reactions these organisms were identical with meningococci. When tested with a meningococcus serum, both were agglutinated, one up to 1 : 100 and the other up to 1 : 20. This they regarded, after making absorption tests, as due to group agglutination. When the serum was saturated with their organism no agglutinin was removed for an undoubted meningococcus; but when the serum was saturated with the meningococcus it was incapable of agglutinating their new strains. Testing these two strains with Dopter's para-meningococcus serum, they found that both were agglutinated, one up to 1 : 500 and the other up to 1 : 150. When this serum was saturated with one of their strains they found it lost its property of agglutinating the para-meningococcus of Dopter. They prepared a serum from a rabbit by inoculations with the strain which only agglutinated up to 1 : 150 with Dopter's para-meningococcus serum. This rabbit serum gave a titre of 1 : 400. It failed to agglutinate undoubted meningococci, but agglutinated Dopter's para-meningococci.

From these experiments they concluded that their two new strains were para-meningococci, but of a variety different from those previously described by Dopter, which were not agglutinated by meningococcus serum.

Dopter and Pauron (20, vi. 1914)<sup>2</sup> reported that they had made further investigations on para-meningococci, with a view to differentiating them from true meningococci by means of absorption tests. They found that some strains of para-meningococci were agglutinated by meningococcus serum, though the majority were not. Para-meningococcus serum very frequently agglutinated meningococci as well as para-meningococci. But the differences between these two groups of organisms became clear when absorption tests were employed. Para-meningococcus serum, saturated with meningococci, lost its agglutinin for meningococci, but retained its agglutinin for para-meningococci; the same serum, saturated with para-meningococci, lost its agglutinin for para-meningococci, but retained its agglutinin for meningococci. Meningococcus serum, saturated with meningococci, lost its agglutinin for meningococci, but retained its agglutinin for para-meningococci.

<sup>1</sup> *C. R. Soc. Biol.* LXXVII, 106.

<sup>2</sup> *Ibid.* LXXVII, 157.

These statements were supported by a table showing tests, after absorption, with a meningococcus and a para-meningococcus serum upon selected strains of meningococci and para-meningococci. It was found that, before absorption, each of the strains was agglutinated by both sera. The number of strains was seven, viz., 4 meningococci and 3 para-meningococci. After absorption, all the tests were either completely negative or definitely positive up to 1:600. Thus, the para-meningococcus serum, when saturated with meningococci, gave 0 with the 4 meningococci and + with the 3 para-meningococci; when saturated with para-meningococci it gave exactly the converse. And precisely similar results, *mutatis mutandis*, were obtained in the saturation experiments with the meningococcus serum.

Dopter and Pauron (27, VI. 1914)<sup>1</sup>, in a subsequent paper, divided their para-meningococci into groups by means of saturation tests. They dealt with 7 strains of para-meningococci, designated S, W, H, B, M, L, Z.

A horse was immunised, at first with S alone. The serum agglutinated S and 3 others, but failed with the other 3. Then the horse was inoculated with the strains which had not agglutinated. The result was a serum which agglutinated all 7, up to 1:400 or 1:600. With this serum saturation experiments were performed.

(1) After saturation with S, all agglutinin was removed for S, W, H, and B, but agglutinins remained (up to 1:400) for M, L, and Z. The results were the same when W, H, and B were used for saturation.

(2) After saturation with M, all agglutinin was removed for M and L, but agglutinins remained (up to 1:400 or 1:600) for the other 5 strains. Saturation with L gave the same result.

(3) Saturation with Z removed agglutinin for Z only, the other six being positive up to 1:400 or 1:600.

The above demarcation into three groups was confirmed by cross-agglutinations with the serum of rabbits vaccinated against each of the 7 strains. Thus: (1) the sera of rabbits vaccinated with S, W, H, and B, agglutinated these 4, but failed with the remaining 3; (2) sera from M and L agglutinated M and L only; (3) the serum from Z agglutinated Z only.

They proposed to call the above three groups the  $\alpha$ ,  $\beta$ , and  $\gamma$  varieties of the para-meningococcus, and suggested that perhaps additional varieties might subsequently be found. They stated that, of the 3 varieties,  $\alpha$  was the commonest and  $\gamma$  the rarest; but I was unable

<sup>1</sup> C. R. Soc. Biol. LXXVII, 231.



to find figures giving the total numbers of each which they have identified. Comparing minor differences as regards cultural growth in these 3 groups, they declared that  $\alpha$  was apparently characterised by a growth like that of the meningococcus, as well as by its group-agglutinability by anti-meningococcus serum;  $\beta$  appeared to differentiate itself from the two others by producing a very viscid growth, heaped up above the surface of the medium, and it also appeared to be more resistant;  $\gamma$  gave a drier type of culture and its colonies appeared flatter.

#### *Discussion of Literature on Serum Reactions.*

The most important feature of the literature summarised above is that it gives evidence of substantial progress in a definite direction.

At first it was thought, despite the recognised trouble of agglutination work with other important cocci, that the agglutination test was going to be a simple matter, providing a definite and unequivocal answer to the question whether any given Gram-negative diplococcus was or was not a meningococcus.

Then a wave of scepticism supervened, because it was found that some strains of undoubted meningococci were not agglutinated by the standard serum employed. Investigation of this difficulty served at first to bring to light further complications, rather than to clear the ground, and, indeed, it induced some observers to take not merely a sceptical but a pessimistic view of the value of agglutination work. But their laboratory data, as a frank statement of difficulties encountered, have aided progress, because they have shown (1) that, with so delicate an organism as the meningococcus, relatively slight differences in technique or condition of the culture may produce apparently wide discrepancies in results; (2) that the idiosyncrasies of individual strains of undoubted meningococci must be thoroughly worked out with monovalent sera; (3) that, until this is done, the resort to polyvalent sera, obviously actuated by the laudable desire for practical expediency, tends to mask the main scientific problem rather than to eliminate difficulties.

More or less concurrently with this output of work by observers whose conclusions practically amounted to the opinion that, in their experience, agglutination tests were unreliable, there was another trend of research, made by men who adhered, as an article of faith, to the doctrine that a really good and, preferably, polyvalent serum



must be infallible, and that any meningococcus-like organism which failed to show specific agglutination with this serum must be stigmatised with some such prefix as "pseudo," "S.," or "para." In its inception, this idea, or working hypothesis, was prompted by the thoroughly sound scientific principle that a meningococcus-like organism found in the naso-pharynx, particularly if found in the naso-pharynx of a non-contact, must be put through its paces very scrupulously before it should be accepted into the order of true meningococci, capable of producing cerebro-spinal fever. But, in the course of further work on the serum reactions of these doubtful organisms, the differences of some of them from undoubted meningococci were not sharp or decisive; evidence of relationship had also to be conceded, and finally it had to be admitted that certain "para" organisms were identical in every respect with strains proved to have been the cause of cerebro-spinal fever.

These facts have cleared the ground by showing that there is a way of reconciliation between these latter results and the laboratory data of the investigators who were sceptical about the reliability of agglutination tests even with meningococci from cerebro-spinal fluid. It has become evident that serological tests have, at least provisionally, placed meningococci into a variety of groups, not, as yet, defined in respect either of number or inter-relationship; and that the criterion for a doubtful organism isolated from the naso-pharynx must be widened accordingly, so as to embrace (1) conformity with one or other of these groups, or (2) failure to conform with any of them.

Taking in detail the laboratory data furnished by individual investigations:

The technique employed by Lingelsheim in 1906 is, in its two essentials (adoption of the macroscopic method and incubation of the dilutions overnight), identical with that which has been followed by the majority of subsequent observers. The two monovalent sera which he prepared appear to have been almost equally satisfactory in agglutinating all the strains tested with them; so there is no evidence from his work that the outbreak in Upper Silesia from which they were derived was due to more than one type of meningococcus. As neither his cerebro-spinal strains nor his strains from the throats of contacts appear to have given any indication that sera such as he employed might not serve as a universal criterion for the diagnosis of the meningococcus, a tribute must be paid to the shrewdness of his remark that, in the case of meningococcus-like organisms obtained from non-contacts,

it must be left for future research to determine whether failure to agglutinate with the particular serum employed justifies the labelling of such organisms as "pseudo."

Kutscher's work at Berlin in 1906 is particularly important from an epidemiological point of view in that he demonstrated the meningococcus in the throats of persons who had not been in contact with the disease. This work was done during the winter 1905-6, at a time when Berlin had remained completely free from the disease during a period of six months. The laboratory details of his work show that it is thoroughly reliable and that the identity of his throat organisms with true meningococci was confirmed by serological tests.

His work with Hübener in 1907 strengthens the importance of these observations, and the significance of the above results is further increased, particularly from the epidemiological aspect, by the findings, based on a much larger number of cases, which were published by Mayer and others in 1910 (p. 427).

The laboratory data published by Eberle in 1908 are of great value in showing (1) the importance of prolonged incubation of the suspensions used in agglutination tests; (2) the fact that an individual cerebro-spinal strain shows different degrees of agglutinability with different specific sera; (3) the fact that different cerebro-spinal strains show different degrees of agglutinability with the same serum. Obviously, these difficulties required further investigation before a throat coccus could be excluded from the group of meningococci because it failed to agglutinate with a particular anti-meningococcus serum.

Arkwright's technique (1909) differs from that of Lingelsheim, Kutscher, and the majority of other observers. He employed the microscopic method, at room temperature, and completed his observations at the end of two hours. Hence it is impossible to make exact comparison between his results and those obtained with the more usual technique. The difference in technique is particularly important because several investigators have shown that a period of two hours, even with incubation at 37° C., is not enough to bring out to the full the specific agglutinative capacities of the serum tested. This difficulty, however, does not obscure a very important feature of Arkwright's work; he has shown clearly that different strains of meningococci obtained in this country differed very markedly in their agglutinability with the sera with which they were tested.

His experiments on complement fixation, in 1911, also illustrate differences between different strains of meningococci.

Elser and Huntoon (1909) differ from many other observers in their method of performing agglutination tests. They incubated their suspensions for two hours at 37° C., and then kept them in the cold (9° C.) for 22 hours, the final reading being taken 24 hours from the start. In support of this method they refer to their comparative experiments on the influence of incubator, room, and ice-box temperature. These experiments (pp. 418-422) fail, in my opinion, to justify a general statement that their method brings out agglutinability as well as incubating for 24 hours, or overnight, at 37° C. or 55° C.

Other observers have found that, when working with such a delicate organism as the meningococcus, results sometimes fail to tally on testing the same strain with the same serum upon different occasions. But Elser and Huntoon go much further; they state that the instability of the meningococcus is very pronounced and constitutes one of the great obstacles to the work. It must be left an open question whether these difficulties would have remained so great if the technique of other observers had been followed.

In their "conclusions" they make the general statement that "approximately 40 % of the meningococcus strains studied were relatively inagglutinable"; and they explain elsewhere that they mean by "inagglutinable" failure to react to powerful sera in 1 : 100.

Turning to their laboratory data for recorded evidence in support of this general statement, one finds that a serum prepared from their strain M 30 was tested with 65 strains and failed to give specific agglutination with 25 of these. This experiment is interesting and important, but insufficient, *per se*, to justify the above broad generalisation. A strain of meningococci may not be agglutinated by one serum, but that does not prove that it is insusceptible to specific agglutination by any other serum.

Seeking further evidence, one finds that experiments were made with monovalent sera prepared from other strains than M 30, and that with these sera, again, some strains were agglutinated but not others.

Whilst recognising that this is corroborative evidence, it must be pointed out that the serological experiments which they have recorded, taken *in toto*, do not suffice to provide definite answers to the three questions: (1) Is it proved that a strain found to be agglutinated by a serum prepared against one meningococcus will necessarily be agglutinated by other sera of high titre? (2) Is it proved that a strain inagglutinable by one serum will necessarily be found inagglutinable by other sera? (3) Is there any ground for supposing that a meningococcus



when tested with two different anti-meningococcic sera, each of high potency with its homologous strain, may give good agglutination with the one, but fail to agglutinate with the other? Elser and Huntoon's data, in so far as they go, would indicate that the answers to questions (1) and (2) are to be "yes," and the answer to question (3) is to be "no." Other observers, working, admittedly, with different strains, have answered questions (1) and (2) by "no" and question (3) by "yes."

Elser and Huntoon make the observation, confirmed by other bacteriologists, that a meningococcus may produce a serum which is a relatively feeble agglutinator for the homologous strain, but produces higher, and undoubtedly specific, agglutination with other strains. But whilst nearly all other observers treat this as no more than a relatively infrequent idiosyncrasy of the meningococcus, Elser and Huntoon regard it as very frequent and highly important. In fact they give the reader the impression that 40 % of their strains belong to the group which are "inagglutinable" but "agglutinogenic." On searching for their recorded laboratory data in support of this view, I find (p. 422) that they are extremely scanty and by no means justify such a sweeping generalisation. In fact the number of strains shown to be agglutinogenic as well as inagglutinable is so small that this apparent discrepancy between Elser and Huntoon's results and those of other observers disappears.

Their experiments with six strains of "pseudo-meningococci," whilst showing differences between these and some of their meningococci, have not been carried far enough to justify the exclusion of the former from the class of meningococci as a whole.

Mayer (1909) maintained that an organism isolated from the nasopharynx could not be regarded as a true meningococcus unless, in addition to conformity in cultural and fermentation tests, it was agglutinated by a good serum, such as the Höchst, up to 1:500. This criterion errs on the side of exclusiveness; no one serum, whether monovalent or polyvalent, has been found which will agglutinate without fail all strains of undoubted meningococci derived from cerebro-spinal fluid.

The work of Friese and Müller (1909), Lieberknecht (1909), and Sachs-Müke (1911) is of great importance in its bearing on the question whether persons not known to have been in contact with the disease may be carriers of the meningococcus. They all found what they called "pseudo-meningococci" or "S." cocci; Friese and Müller found them in the throats of soldiers during the prevalence of an epidemic regarded



as influenza; Lieberknecht, in normal school children; and Sachs-Müke, in normal soldiers. From the laboratory data of their work which I have given, it is evident that they did their best to prove that these organisms were not true meningococci, but that they were compelled to admit a close kinship. They have not succeeded in proving non-identity, because the criteria on which they relied would also exclude some strains of meningococci which have been proved to be the cause of cerebro-spinal fever. I refer in particular to their assumptions (1) that one particular anti-meningococcic serum, if polyvalent and of high titre, must be infallible; (2) that a strain is not a meningococcus if its behaviour in serological reactions is not identical with one or two examples of undoubted meningococci; and (3) that there is a specific difference between true meningococci as a class and "pseudo-" meningococci as a class in respect of their comparative agglutinability at 55° C. and 37° C. As regards this last point, it must be pointed out that Kutscher, who was the first to show the advantages of incubation at 55° C. in enhancing the agglutinability of certain strains, denies (1912)<sup>1</sup> that specific differences are brought out between true and "pseudo-" meningococci by comparing agglutinability at 37° C. and 55° C. He concludes his criticisms of the efforts of the above authors to distinguish their throat strains from true meningococci with a verdict of "not proven."

When Dopter, in 1909, found organisms in the naso-pharynx which differed from his strains of undoubted meningococci only in respect of certain serological reactions, he was too sound a pathologist to stigmatise these as "pseudo" (*i.e.*, not specifically pathogenic for man); he explicitly stated that he thought they might be capable of producing cerebro-spinal fever. But to distinguish them from his fully accredited meningococci he gave them the prefix of "para." In 1911 he was able to give his para-meningococci their full credentials, by producing instances where cerebro-spinal fever had been caused by them; and in 1912 he reported that the number of such cases which he had collected amounted to twelve.

The great interest and importance of this work is that it goes far beyond the stage when, though it was recognised, willingly or unwillingly, that strains of meningococci did not all agree in serological reactions, these differences were treated merely as inconvenient idiosyncrasies which should be neutralised, as far as possible, by the production of polyvalent sera. Dopter showed that serological tests

<sup>1</sup> Kolle und Wassermann, *Handbuch der path. Mikroorganismen*, 2nd ed., iv.

brought out what appeared to be group distinctions between different strains of Weichselbaum's diplococcus, the smaller of the two groups to which he called special attention being distinguished from the larger by the somewhat irksome prefix "para."

In 1914 this "para" group received further attention at the hands of Darré and Dumas, and Dopter and Pauron; and some differences were found between individual members of it. Perhaps the sub-division of the group, by Dopter and Pauron, into  $\alpha$ ,  $\beta$ , and  $\gamma$  varieties should be regarded as merely provisional. If the authors devoted equal endeavour to the discovery of possible differences in their larger group, which I may call "ortho" as a convenient distinction from "para," they might again find distinction between individual members, tempting to another sub-division into varieties; or they might even find a strain which could not properly be called either "ortho" or "para," but demanded a third group. In short, it seems to me that it would be premature to take the division into "ortho" and "para" as completely comprehensive and final, and then try to force any and every meningococcus into one or other of these groups. The meningococci are a large family and many strains must be worked out, each on its own merits and irrespective of any preconceived idea of grouping, before it can be settled what are the distinctive features, each common to several members and confined to these members, and whether it is possible to establish a system of grouping without overlapping or cross-division. Before this is done, sub-division into  $\alpha$ ,  $\beta$ , and  $\gamma$  varieties is likely to lead to confusion.

#### THE INVESTIGATIONS IN THE BOARD'S LABORATORY.

##### (1) *Meningococci from Cerebro-spinal Fluid.*

The Board's Laboratory has received for diagnosis specimens of cerebro-spinal fluid withdrawn by lumbar puncture from patients, in various parts of England and Wales, whose disease was suspected to be cerebro-spinal fever. Cultures from 16 of these cases have been utilised by Dr Griffith and me in the present investigation. We are also indebted to Dr Nabarro who has supplied us with 7 additional strains (5 from Great Ormond Street Hospital and 2 from private cases); to Dr Forbes, of the London County Council, who has sent us 7 strains; and to Dr Caiger, of the South-Western Fever Hospital, who has supplied us with 4 strains, through Dr W. M. Scott.

The details of Dr Scott's work on cerebro-spinal strains are given in his report (pp. 464-484).

As the cultural characters of the meningococcus are well known, Dr Griffith and I consider that a brief description of our observations and technique will suffice. We used for plating our material Kutscher's serum-agar medium, the special features of which are that the serum is fresh ox serum and the agar is made with broth from human placentas. The day after inoculation the colonies appear as translucent, shiny, bluish grey, raised, convex discs, of circular outline and from about  $\frac{1}{2}$  to  $1\frac{1}{2}$  mm. in diameter. Under low magnification they appear homogeneous, or only very finely granular, and have a clear periphery. When touched with a platinum needle, they are found to be slightly viscid, not coherent and not friable. They emulsify readily. On the second day the colonies are larger (1 to  $2\frac{1}{2}$  mm.), semi-opaque, with a narrow, clear periphery. The colonies are not pigmented, but the central portion of the older colonies may appear creamy or yellowish in contrast to the grey colour of the thinner peripheral zone. Microscopically, the organisms are Gram-negative diplococci, many of which are flattened along their apposed surfaces; their size is variable, some being conspicuously larger than the rest and more deeply stained; tetrads are usually found. On plain agar slants, sub-cultures from a primary colony usually give no growth, but if the tube be thickly inoculated a few colonies may develop; from later generations a fairly good growth may be obtained. At 22° C., there is no growth on gelatine; on nutrose- (or glucose-) ascitic agar there is usually no growth, but if the tube be richly inoculated there is sometimes a scanty growth, in the form of discrete colonies; on Kutscher's medium growth may fail, but a scanty growth is more frequently obtained; on egg it is the rule to obtain a growth at this temperature, usually in the form of a thin, shiny layer. The coccus rapidly dies out in unsealed tubes of Kutscher's medium or ascitic agar, but in sealed egg tubes it remains alive for several weeks.

For the fermentation tests we used Lingelsheim's solid media (ascitic agar containing 1 % of the sugar and coloured with litmus). We used five sugars, viz., glucose, maltose, galactose, laevulose, and saccharose. We never obtained indication of acid formation with any of the last three; but there was always acid formation with both glucose and maltose. Some strains produced stronger fermentation with one of these sugars than with the other.

Further observations on the fermentation tests and also the work on



the serological relations of our cerebro-spinal strains are recorded in Dr Griffith's report (pp. 446-463).

(2) *Examination of Naso-pharyngeal Swabs from Non-contacts.*

(a) *Patients at St Bartholomew's Hospital.*

For this investigation the Board obtained the assistance of Mr C. E. West, F.R.C.S., Aural Surgeon to St Bartholomew's Hospital, who made arrangements for the examination of patients not known to have been in contact with any cases of cerebro-spinal fever.

The majority of the persons swabbed were out-patients attending the Aural Department; the rest were from other departments of the Hospital, some being wounded soldiers and the remainder civilians under treatment for one or other of a variety of medical and surgical diseases not affecting the naso-pharynx.

The persons examined were taken as general examples of hospital patients, without any selection according to their clinical condition. When pharyngitis was observed, a note to this effect was recorded.

Freshly poured plates were sent from the Board's Laboratory to the Hospital, where Mr West, or in some cases his house surgeon, took the swabs and immediately inoculated a plate with each. Within from one to three hours after the swabbing the plates were received in the Laboratory, together with notes of each case.

Dr F. Griffith and I then proceeded with the bacteriological investigation. The plates (Kutscher's medium) were spread at once with a bent glass rod, which was transferred to a second plate, and, when much mucus was present, from a second to a third. The time elapsing between swabbing the patient and incubating the plates did not exceed three hours. Colonies resembling meningococci macroscopically, microscopically, and in readiness of emulsification were put through the same cultural and fermentation tests as the cerebro-spinal strains.

Cultures of 502 swabs from the naso-pharynx were examined. Of these, the following are excluded in the appended tabulation of results:—8 which rapidly become overgrown; 10 which were repeats from patients previously cultured and found positive; 1 (positive) where it was found that the patient had been in contact with a case of cerebro-spinal fever; 1 (positive) where the patient gave a vague history of an illness, 3 months previously, which might have been meningitis; 2 where, on sub-culture, the cultural and fermentation reactions appeared typical at first, but yellow pigmentation subsequently



developed. Of the remaining 480, 49 yielded cultures which resembled meningococci microscopically, culturally, and in fermentation tests.

*Cultural Tests of Naso-Pharyngeal Swabs.*

Age period	Males		Females		Totals (male and female)	
	Positive	Negative	Positive	Negative	Positive	Negative
0- 5 years	0	13	1	8	1	21
5-10 „	4	38	0	15	4	53
10-20 „	8	62	3	69	11	131
20-40 „	17	78	6	79	23	157
Over 40 „	7	42	3	27	10	69
Totals ...	36	233	13	198	49	431
	13.4 % positive		6.2 % positive		10.2 % positive	

With reference to the interpretation of these statistics, attention must be called to the following special circumstances:—(1) The persons investigated, being hospital patients, were not representative of the normal, healthy population. (2) The swabs were taken by an aural surgeon who was expert in obtaining naso-pharyngeal swabs free from contamination by mouth organisms; so the risk of any meningococcus-like organisms present being overgrown was reduced to a minimum. (3) The medium (Kutscher's) on which the swabs were plated is especially favourable for the development of meningococcus-like organisms and for their identification. (4) In examining the plates we were more fortunately placed than bacteriologists who are required to diagnose as many cases as possible in as brief a time as possible. We never received more than 20 cases in one day, or more than about 50 in a week, and had time to make minute and repeated examination of each plate inoculated.

For the above reasons, our percentages of positives are, perhaps, above the average.

The collection of the above 480 samples commenced on March 29th, 1915, and terminated on July 22nd of the same year. The first 100 (completed April 19th) gave 20 positives; the second 100 (completed May 6th) gave 7; the third 100 (completed June 7th) gave 6; the fourth 100 (completed June 24th) gave 7; the last 80 (completed July 22nd) gave 9.

The "repeats" of previous positives were too few to justify any general conclusions. Two out of the ten were positive, one after an interval of 28 days from the first swabbing and the other after an interval of 61 days.

On comparing the positive and negative results with the clinical notes, it is not found that a conspicuously large number of the positive cases showed pharyngitis when the swab was taken.

Further work on the identification of these meningococcus-like organisms, with special reference to their serological reactions, was undertaken by Dr F. Griffith and is recorded in his report (pp. 446-463).

(b) *Investigations by Dr Scott.*

Dr W. M. Scott, working in the Board's Laboratory independently, but on parallel lines and employing the same technique and culture media, examined naso-pharyngeal swabs from 194 non-contacts, consisting of 138 persons attending the out-patient department of the Lambeth Infirmary, and 56 children attending a rural school in Kent. His results are recorded in his report (pp. 464-484).

CONCLUSIONS.

It has been definitely established that organisms indistinguishable from meningococci by microscopic, cultural, and fermentation tests occur in the naso-pharynx of some persons who, so far as is known, have not been in contact with cases of cerebro-spinal fever.

The question whether this apparent identity can be confirmed by serological tests raises the wider problem:—what are the serological criteria to which strains of undoubted meningococci conform? This problem is far from being completely elucidated. It has, however, already been made clear that differences exist between the reactions of different strains, and that these differences appear to separate out the majority of the strains into different groups. But it is still, in my opinion, an open question to what extent and under what principles a permanent grouping can be established.

In considering the principles of classification, one must endeavour to determine distinctive group characteristics and, at the same time, to discriminate between such characteristics and minor distinctions which might, perhaps, offer a basis for sub-grouping. Taking the experimental work in the order of laboratory procedure, there are, at once, three important characteristics which require consideration. The first step is to immunise an animal with a particular culture; the second to see what strains are agglutinated by the serum prepared; and the third, when necessary, to ascertain by absorption tests if the

agglutination is specific. Correspondingly, the three characteristics to be considered as criteria are:—(1) agglutinogenic capacity; (2) agglutinability; (3) absorptive capacity. If each individual strain of meningococci behaved consistently in respect of these three criteria, there would be a substantial basis for the grouping of different strains. But if individual strains differ in the above respect, grouping under these three criteria would lead to cross-division. Such cross-division could only be avoided by making one of these criteria absolute, and relegating the other two to the minor function of criteria for sub-grouping. In my opinion, enough work has not yet been done to justify the establishment of criteria for the demarcation into hard-and-fast groups; and, in view of this consideration, I think it would be premature to adopt a particular standard and then attempt to dispose of discrepancies from it by elastic expansion into sub-groups.

It is likely to be a long time before the question of classification is finally settled. In the meantime, comparative serological tests are available between individual cerebro-spinal strains and strains of naso-pharyngeal origin. It has been found that some of the latter, derived from non-contacts, coincide with certain cerebro-spinal strains, and that other non-contact strains, though not coinciding precisely, show serological relationship to cerebro-spinal strains.

How far do the above considerations provide an answer to the question: is a naso-pharyngeal strain, found identical with true meningococci in cultural and fermentation tests, to be regarded, *ipso facto*, as capable of producing cerebro-spinal fever in a suitable soil? It is impossible to reply by a categorical "Yes" or "No," because the evidence is incomplete. The balance of available evidence is on the side of "Yes." Is future work likely to reverse that balance? It might, if it can be shown that serum reactions or animal experiments place the great majority of non-contact strains into a distinct class. But this evidence is not yet forthcoming. If it is provided in the future, it will be necessary for me to reconsider my present opinion, which is that all naso-pharyngeal strains, as defined above, should be regarded as possibly capable of producing cerebro-spinal fever.

## IDENTIFICATION OF THE MENINGOCOCCUS IN THE NASO-PHARYNX WITH SPECIAL REFER- ENCE TO SEROLOGICAL REACTIONS.<sup>1</sup>

By FRED. GRIFFITH, M.B.

IN view of the apparent identity, so far as morphological and cultural characters are concerned, of meningococci from cases of cerebro-spinal meningitis with certain gram-negative cocci found in the naso-pharynx of non-contacts, it has been necessary to resort to serological tests to obtain further information on the question of their inter-relationship.

Monovalent agglutinating sera have been prepared from several varieties of each class of cocci and cross agglutination experiments have been made. The tests have been applied to cerebro-spinal strains and to naso-pharyngeal strains isolated during the recent epidemic. The strains used are those mentioned in Dr Eastwood's Report (pp. 405-445), which have been jointly investigated by us as regards cultural characters and fermentative capacities. I have submitted to agglutination tests the 34 cerebro-spinal strains and 30 of the naso-pharyngeal strains from patients at St Bartholomew's Hospital not known to have been in contact with cases of cerebro-spinal meningitis.

Before proceeding to the work on agglutination, I record additional observations on the cultural and fermentative capacities of the strains investigated, with special reference to differential features on various media. For assistance in the arrangement of the subject matter of this report, I am greatly indebted to Dr Eastwood.

### FURTHER OBSERVATIONS ON CULTURAL CHARACTERS AND FERMENTATION TESTS.

In examining the primary cultures on placental serum agar plates, slight differences were noted between the meningococcus colonies from the cerebro-spinal fluid and the meningococcus-like organisms

<sup>1</sup> Reprinted by permission of H.M. Stationery Office, from *Reports to the Local Government Board on Public Health and Medical Subjects*, n.s. No. 110 (1916).—ED.



from the naso-pharynx. These differences, consisting mainly of slight variations in opacity, were eliminated when colonies of either class were plated in pure culture on fresh medium. Changes in shape and structure of single colonies were common to both classes of cocci as they increased in age. In fact, all slight variations in colour, consistency, ease or difficulty of growth, fermentative activity, or viability of the cerebro-spinal strains were also met with among the naso-pharyngeal strains, but none was found to be a feature distinguishing the one class from the other.

The fermentation tests of various sugars are next in order of importance to the appearances of the primary colonies as a means of identifying the meningococcus. Maltose and glucose only showed evidence of acid fermentation. Meningococci, recently isolated from cerebro-spinal fluid, rarely produced equal amounts of acidity from glucose and maltose contained in Lingelsheim's solid sugar media. They could, with a few exceptions, be placed in one or other of two groups, according as they fermented glucose or maltose more strongly, and, as will be seen in Table I, the two groups were correlated with the serological grouping. In the case of the naso-pharyngeal strains, on the other hand, both sugars were more often equally fermented (Table II). In fact, this feature was so noticeable that, in the early part of the work, it was considered as a possible means of distinguishing the so-called pseudo-meningococcus from the true meningococcus. But further observations showed that some strains of meningococci, recently obtained from cerebro-spinal fluid, fermented maltose and glucose equally. The acidity with either of the two sugars was sometimes extremely slight in degree and evanescent. The above observations, recorded in Tables I and II, refer to tests on strains recently isolated. On repeating the tests after prolonged sub-cultivation, the differences at first noted were in many cases found to have disappeared, and in one case even to be reversed.

On egg meningococci grow readily, producing a smooth, shiny layer slightly pink at the bottom of the tube, the same tint appearing in the growth when heaped up. Cultures remain alive in sealed tubes of this medium for many weeks and have been sub-cultivated after seven months in the incubator at 22° C.

Glucose agar was found to be a useful medium for producing considerable quantities of culture; it has the advantage of giving a firm surface which facilitates the removal of the growth with a platinum spatula. It was difficult to induce some strains to grow on

this medium, when first transferred from one containing serum; in this respect some of the naso-pharyngeal strains were found to be the most obstinate. With such strains the early growths produced on the glucose agar were very sticky. In preparing this medium, in order to avoid unfavourable changes in reaction, it is advisable to add the sugar, sterilised in distilled water, after the final sterilisation of the nutrient agar.

#### TECHNIQUE OF AGGLUTINATION TESTS.

The sera were prepared by inoculating intravenously adult rabbits with increasing doses of living culture grown on glucose agar. The dose ranged from  $\frac{1}{8}$  tube of culture to  $1\frac{1}{2}$  tubes, which was found to be as much as could be given without killing the animal. The inoculations were usually made at intervals of 8–10 days, but no regular routine was followed in this respect. The progress of immunisation was followed by making frequent tests of small quantities of blood serum withdrawn from an ear-vein. Inoculations were repeated until a workable serum was produced, the time necessary to this end varying, with different rabbits, from 4 weeks to 14 weeks.

The culture suspensions were standardised in the following way. The moist growth on several glucose agar tubes of 24 hours' incubation was removed and weighed on a chemical balance. After emulsifying in .5 % carbolic acid in normal salt solution, and heating to 65° C. for an hour, the suspensions were diluted to a uniform strength of 4 mg. per cubic centimetre, and were stored in rubber-capped tubes in a cool dark place.

The agglutination tests were made in tubes measuring 3 in. by  $\frac{1}{2}$  in. To each .5 c.c. of serum dilution, .5 c.c. of culture suspension was added and the tubes were placed in the incubator at a temperature of 55° C. for 24 hours, when the first readings were taken. They were then removed to the ice-chest and the final results were noted the following morning.

In order to present the results in a convenient manner, since it was impossible to give all the tables in full, it was necessary to select an end point. I have chosen as my end point the highest dilution in which the cocci had clumped and sedimented, leaving the supernatant fluid clear, or with the faintest suspicion of turbidity. This is represented in the tables by a figure giving the numerical value of the dilution. When the turbidity, though slight, was distinct and agglutination was

well marked, the result is represented by the symbols  $\pm$  or  $\mp$ , according to the degree of turbidity,  $\pm$  meaning agglutination well marked but incomplete in 1 in 50,  $\mp$  meaning agglutination slight but definite. The negative sign indicates that the reaction was completely negative in 1 in 50, the highest concentration of serum considered, or showed no more than a trace of agglutination.

In order to compare agglutination with fermentative activity of individual strains, I have inserted in each of the tables a column showing the relative activity of each strain upon maltose and glucose.

In making orientation tests with a view to determining from which strains to prepare serum, I first selected two strains of cerebro-spinal origin, M. 8 and M. 23, the former because it fermented maltose more than glucose and the latter because its action on these two sugars was the reverse. On testing several strains of meningococci with these two sera I found obvious differences in agglutinability which appeared to coincide with differences in fermentative capacity. The subsequent selection of strains for the preparation of sera was determined by the differences in agglutinability already observed. At this stage it was found that the majority of the strains tested were agglutinated by one or other of the five sera, M. 8, M. 23, M. 9, M. 24, and M. 28. With a few of the strains, however, none of the sera gave good agglutination, and two of these last strains, M. 17 and M. 32A, were selected for the production of immune sera. On completing my results I re-arranged my strains in the order which exhibited most clearly their differences in agglutinability.

#### ANALYSIS OF TABLE I.

Table I gives the results of agglutination tests performed upon 34 strains of meningococci, with sera prepared from 7 of the same, and from 3 meningococcus-like organisms of naso-pharyngeal origin. The test with the first serum, M. 8, shows that this divides the meningococci into agglutinable and inagglutinable strains. That the inagglutinability is only relative to the particular serum employed will be seen on examination of the next column, where the series is tested with serum prepared from one of the strains, M. 23, not agglutinated by M. 8 serum. So far the results appear to justify the division of the meningococci into two main groups—Group I from M. 1 to M. 17, and Group II from M. 18 to M. 31. The remaining four strains, two of which, M. 32 and M. 32A, came from the same patient after an interval of 15 days, can be included in neither of the two groups, since in three, agglutination

TABLE I.

*Cross-agglutination tests with 34 strains of meningococci and mono-valent sera prepared with 7 cerebro-spinal fluid strains and 3 naso-pharyngeal strains of Gram-negative cocci:*

Strain	Fermenta- tion of glucose and maltose	M. 8 Serum	M. 23 Serum	M. 9 Serum	M. 24 Serum	M. 28 Serum	M. 17 Serum	M. 32A Serum	N.P. 11 Serum	N.P. 10 Serum	N.P. 26 Serum	Normal Rabbit Serum
M. 1	M. > G.	1000	—	1000	—	—	100	±	400	±	±	—
M. 2	M. > G.	1000	±	1000	∓	∓	200	—	500	50	±	—
M. 3	M. > G.	800	50	1000	±	—	200	—	500	±	±	—
M. 4	M. > G.	800	50	800	∓	±	200	—	400	50	±	—
M. 5	M. > G.	800	±	800	—	∓	100	—	400	±	—	—
M. 6	M. > G.	600	±	800	—	—	100	∓	400	±	∓	—
M. 7	M. > G.	500	50	500	50	—	100	—	300	—	∓	—
M. 8	M. > G.	400	±	400	50	±	100	±	200	±	±	—
M. 9	M. > G.	400	∓	400	±	±	100	—	400	—	±	—
M. 10	M. > G.	200	—	300	—	—	200	—	400	—	—	—
M. 11	M. > G.	200	±	400	100	±	100	±	600	±	—	—
M. 12	Equal	100	—	400	±	—	800	—	400	—	—	—
M. 13	M. > G.	100	50	400	±	∓	400	∓	400	—	±	—
M. 14	M. > G.	100	∓	400	50	—	100	—	400	±	—	—
M. 15	M. > G.	100	—	400	50	—	100	∓	400	—	±	—
M. 16	M. > G.	100	—	100	50	100	—	50	50	±	∓	—
M. 17	Equal	50	∓	100	∓	∓	400	∓	400	—	—	—
M. 18	Equal	—	1000	—	800	500	—	50	100	400	50	—
M. 19	G. > M.	∓	1000	±	800	400	200	400	∓	400	±	—
M. 20	G. > M.	∓	800	—	800	300	—	100	50	800	100	—
M. 21	Equal	100	800	∓	1000	500	—	300	∓	500	100	—
M. 22	G. > M.	∓	600	—	400	500	—	400	300	200	200	—
M. 23	G. > M.	—	500	—	100	400	—	400	±	200	∓	—
M. 24	G. > M.	±	500	—	300	500	—	200	±	200	±	—
M. 25	G. > M.	±	400	±	800	200	—	±	±	200	±	—
M. 26	G. > M.	—	400	—	800	300	—	400	100	100	—	—
M. 27	G. > M.	—	400	—	800	500	100	50	±	400	—	—
M. 28	G. > M.	—	400	—	100	500	—	—	±	200	∓	—
M. 29	G. > M.	—	400	∓	800	500	—	50	±	200	50	—
M. 30	G. > M.	—	400	—	800	300	—	100	100	300	±	—
M. 31	G. > M.	—	200	—	100	200	100	50	100	50	±	—
M. 32	Equal	—	±	—	50	∓	±	800	±	—	±	—
M. 32A	Equal	—	±	—	50	∓	—	800	±	±	50	—
M. 33	G. > M.	±	∓	—	100	50	—	50	100	±	100	—
*M. 34	G. > M.	+	+	+	+	+	+	+	+	+	+	+

The symbols ±, ∓, —, refer to reactions at 1:50.

± = Well marked but incomplete agglutination.

∓ = Slight but definite agglutination.

— = No agglutination.

\* This strain possessed the property of auto-agglutination.



is insufficiently marked, and in the fourth, M. 34, is not specific, since agglutination took place in normal salt solution, and with normal rabbit serum.

On examination of the individual strains in Group I, one finds marked differences in the degree to which they are agglutinated by M. 8 serum. Several show no more agglutination than M. 21, which has been included in Group II. In Group II likewise the various strains are unequally agglutinated by M. 23 serum. With M. 17, the least agglutinable of Group I, a serum was prepared and tested on the whole series. It was found to agglutinate, with a single exception, all the individuals in Group I, and, in addition, three in Group II. This shows that, in respect of its agglutinogenic capacity, M. 17 belongs to Group I.

In addition to the above three sera, I have prepared also sera from M. 9, a member of the first group, and from M. 24 and M. 28, members of the second group. In column five, M. 9 serum is markedly specific in its action upon strains in Group I. M. 24 and M. 28 sera agglutinate mainly strains in Group II<sup>1</sup>. These three sera, therefore, corroborate the above division of the strains into Groups I and II.

The two strains of M. 32 and the strain M. 33 may now be considered. From the agglutination tests with the sera prepared with M. 8, M. 23, M. 9 and M. 17, there is no evidence that they are related to one group more than the other, and, indeed, no serological evidence that they are meningococci. But the serum M. 24 was found to agglutinate them slightly. A serum was then prepared from M. 32, which shows that this strain has agglutinogenic capacities establishing its relationship to meningococci of Group II. In preparing a serum from M. 33 (not included in the table), more than usual difficulty was experienced, and up to the present no higher titre than 1 in 100 for the homologous strain has been reached. With this serum several strains in Group II only were agglutinated, in dilutions of 1 in 50 and 1 in 100.

The auto-agglutinating strain, M. 34, was heated to 80° C. for an hour. This treatment apparently destroyed the auto-agglutinating property and a permanent suspension in salt solution could be made, which showed no trace of agglutination in normal rabbit serum in a dilution of 1-10. This suspension of M. 34, tested subsequently with immune sera, gave an agglutination titre of 1 in 1000 with M. 23 serum, 1 in 800

<sup>1</sup> M. 28 was a more active serum than would appear, but owing to an accident to a centrifuge tube only sufficient serum was preserved to make the test on the whole series in dilutions from 1 in 50 to 1 in 500.

with M. 32a serum, and only a slight agglutination in 1 in 200 with M. 8, results which indicate that the strain probably belongs to Group II.

The agglutination tests with the three sera N.P. 11, N.P. 10, N.P. 26, can best be considered in conjunction with the tests given in Table II on the naso-pharyngeal strains.

The salient facts brought out in Table I are (1) that a series of meningococci tested upon a monovalent meningococcus agglutinating serum can be arranged to show a progressive diminution in agglutinability, ending in a number of completely inagglutinable strains; (2) a second monovalent serum will give a similar result, but in inverse order, the inagglutinable becoming the agglutinable.

But when a larger number of sera is employed, this parallelism between (1) and (2) is found not to be absolute, since certain strains which were inagglutinable in (1), but agglutinable in (2), do not always correspond in regard to agglutinability or inagglutinability, respectively, when tested with other sera prepared with members of Groups I and II.

#### ANALYSIS OF TABLE II.

The 30 naso-pharyngeal strains have been tested with 9 of the sera, the agglutinating properties of which have been studied in relation to meningococci of cerebro-spinal origin.

In consequence of the difficulty in obtaining good growths of many of the naso-pharyngeal strains upon glucose agar, a sufficient quantity of culture suspension was not made at one time for the whole series of tests, as was the case with the majority of the cerebro-spinal fluid strains. The different suspensions of the same strain have not been equally agglutinable, and in repeated tests the agglutination results, though in general conformity, have not been always identical.

Applying the same method of analysis as in the consideration of Table I, I find that the two sera, M. 8 and M. 23, each divide the series into an agglutinable and an inagglutinable class. Whereas in the case of the cerebro-spinal meningococci, the strains relatively inagglutinable to both sera were few in number, in this series they comprise the majority. As with the cerebro-spinal meningococci, the agglutinable naso-pharyngeal strains are divided into two groups by the sera M. 8 and M. 23. M. 9 serum also agglutinates only the same four strains as M. 8 serum, thus confirming the almost complete identity of agglutinogenic function already exhibited by the strains M. 8 and M. 9 in relation to the cerebro-spinal fluid strains. Again, M. 24 serum agglu-

tinates the same strains as M. 23 serum, but the agglutinogenic capacity of M. 24 has a wider range, since the serum agglutinates in addition a number of other strains. Still greater agglutinating capacity towards the naso-pharyngeal strains is shown by M. 32A serum. M. 32A is a meningococcus strain, which was agglutinated, to any considerable

TABLE II.

*Cross-agglutination tests with 30 strains of naso-pharyngeal Gram-negative cocci and monovalent sera prepared with 6 cerebro-spinal fluid strains and 3 naso-pharyngeal strains:*

Strain	Fermenta- tion of glucose and maltose	M. 8 Serum	M. 23 Serum	M. 9 Serum	M. 24 Serum	M. 17 Serum	M. 32A Serum	N.P. 11 Serum	N.P. 10 Serum	N.P. 26 Serum	Normal Rabbit Serum
N.P. 1	M. > G.	800	—	1000	±	100	±	400	—	±	—
N.P. 2	Equal	200	±	300	200	100	50	400	100	200	—
N.P. 2A	M. > G.	100	—	50	50	50	±	300	±	50	—
N.P. 3	Equal	50	±	100	±	±	50	200	±	100	—
N.P. 4	M. > G.	50	—	50	300	±	400	100	—	100	—
N.P. 5	Equal	±	±	±	50	100	200	400	—	100	—
N.P. 6	G. > M.	—	800	±	300	—	50	100	200	100	—
N.P. 7	G. > M.	—	400	—	100	—	100	50	100	100	—
N.P. 8	G. > M.	—	400	±	100	—	100	300	200	±	—
N.P. 9	Equal	±	400	±	300	±	400	300	200	200	—
N.P. 10	G. > M.	—	500	—	800	—	±	50	500	50	—
N.P. 11	M. > G.	±	400	±	300	50	50	300	100	±	—
N.P. 12	Equal	±	50	±	100	—	100	100	±	100	—
N.P. 13	M. > G.	±	50	±	50	±	200	400	—	±	—
N.P. 14	G. > M.	50	±	±	200	±	100	400	100	100	—
N.P. 15	Equal	±	±	±	100	±	100	100	—	±	—
N.P. 16	Equal	±	±	±	200	50	200	800	200	400	—
N.P. 17	M. > G.	±	—	...	100	—	200	400	100	400	—
N.P. 17A	Equal	±	—	±	±	—	200	400	±	300	—
N.P. 18	Equal	±	—	±	50	—	200	400	—	300	—
N.P. 19	Equal	±	±	±	50	±	50	300	±	100	—
N.P. 20	Equal	—	—	—	±	—	50	400	—	50	—
N.P. 21	Equal	±	—	±	±	±	±	400	±	100	—
N.P. 22	M. > G.	±	±	±	±	—	100	400	±	100	—
N.P. 23	Equal	±	—	±	±	—	50	400	±	100	—
N.P. 24	Equal	±	—	±	±	—	±	400	—	±	—
N.P. 25	M. > G.	±	—	±	±	±	±	400	—	100	—
N.P. 26	Equal	±	±	±	±	—	200	200	±	400	—
N.P. 27	M. > G.	—	—	±	±	—	±	400	—	100	—
N.P. 28	Equal	—	—	±	100	—	—	±	—	—	—
N.P. 29	Equal	—	—	...	±	—	±	50	...	±	—
N.P. 30	...	—	—	—	—	—	—	—	—	—	—

The symbols ±, ±, —, have the same significance as in Table I.

N.P. = Non-contact naso-pharyngeal strain.

extent, by the homologous serum alone, though the serum prepared from it agglutinated several cerebro-spinal strains of Group II. Thus agglutinogenic capacity demonstrates its relationship alike to the cerebro-spinal meningococci and to the naso-pharyngeal cocci.

N.P. 11 produces a serum which has marked agglutinating properties for almost all the naso-pharyngeal strains, as well as for cerebro-spinal strains in both of the groups. With this strain, however, agglutinability and agglutinogenic capacity are not in correspondence. N.P. 11 is agglutinated by the sera of M. 23 and M. 24, members of Group II, but the serum produced by N.P. 11 agglutinates mainly members of Group I, giving only incomplete reactions in 1 : 50 with M. 23 and M. 24.

N.P. 10 which was agglutinated by M. 23 serum, produces a serum which agglutinates with a few additions the same naso-pharyngeal strains as M. 23 serum, and reference to Table I shows that N.P. 10 serum acts mainly upon the group of meningococci to which M. 23 belongs.

From the above considerations it is clear that there is a definite relationship between the cerebro-spinal meningococci and many of the naso-pharyngeal cocci. Some, however, of the latter strains were agglutinated only to a very slight extent by the six immune sera produced by meningococci of cerebro-spinal origin. The question arises whether it is possible by serological tests to justify a classification of these cocci as meningococci. It has already been pointed out, in analysing Table I, that two strains from cases of meningitis, M. 32 and M. 33, agglutinated only to a minor degree with five meningococcus immune sera, but their identity with meningococci has been confirmed by demonstration of their specific agglutinogenic capacity. Experiments on similar lines have been begun with the naso-pharyngeal strains which are apparently inagglutinable when tested by some of the above meningococcus immune sera. Some progress in this direction has already been attained. With one of these strains, N.P. 26, an agglutinating serum was prepared. This serum agglutinated to some extent the majority of the naso-pharyngeal strains, and (Table I) a number of the meningococci in Group II.

The two strains which differed in certain cultural features from meningococci, N.P. 29 and N.P. 30, have been included as controls. N.P. 29 produced slight yellow pigmentation on glucose ascitic agar, but fermented only maltose and glucose. N.P. 30 was markedly pigmented and fermented in addition laevulose.

The points of importance in the analysis of this table of agglutination tests are (1) that the majority of the naso-pharyngeal strains, fermenting



only glucose and maltose, show some degree of correspondence with meningococci of cerebro-spinal origin as regards agglutinability; (2) a certain number can definitely be pronounced as identical with standard strains of meningococci; (3) when agglutinogenic capacity is included as proof of relationship, further evidence is provided of correspondence between cerebro-spinal meningococci and naso-pharyngeal strains of non-contacts.

#### AGGLUTININ ABSORPTION EXPERIMENTS.

The following experiments have been made with the object of comparing absorptive capacity with agglutinability.

##### *Experiment 1.*

M. 8 serum absorbed with cerebro-spinal and naso-pharyngeal strains.

Each absorbing strain was suspended in 1 c.c. of 1 in 10 dilution of serum. In Table III A the amount of culture added was the whole

TABLE III\*.

Absorbing strain	Dilutions of absorbed serum tested upon homologous strain					Titre of absorbing strain for M. 8 serum before absorption
	40	80	160	320	640	
(Control serum)	+	+	+	+	trace	...
M. 8	trace	—	—	—	—	400
M. 9	±	—	—	—	—	400
M. 3	trace	—	—	—	—	800
M. 4	trace	—	—	—	—	800
M. 16	+	+	±	—	—	100
M. 17	+	+	+	±	trace	50
M. 32	+	+	+	+	trace	—
M. 23	+	+	+	+	trace	—
M. 32 <sub>A</sub>	+	+	+	±	trace	—
M. 33	+	+	+	+	trace	±
M. 24	+	+	+	+	trace	±
N.P. 1	trace	—	—	—	—	800
N.P. 14	+	+	±	trace	—	50
N.P. 4	+	+	±	±	—	50
N.P. 15	+	+	+	+	trace	±
N.P. 13	+	+	+	+	trace	±
N.P. 9	+	+	+	+	trace	±
N.P. 27	+	+	+	+	trace	—
N.P. 17	+	+	+	+	trace	±

\* In this and the following tables—

+ Complete agglutination.

± Marked, but incomplete agglutination.

± Slight, but definite agglutination.

B.						Titre of absorbing strain for M. 8 serum before absorption
Absorbing strain	Dilutions of absorbed serum tested upon homologous strain					
	40	80	160	320	640	
(Control serum)	+	+	+	+	±	...
N.P. 2	+	+	+	±	—	200
N.P. 2A	+	+	±	±	—	100
N.P. 17	+	+	+	+	±	±
N.P. 18	+	+	+	+	±	±
N.P. 20	+	+	+	±	±	—
N.P. 21	+	+	+	±	∓	±
N.P. 22	+	+	+	±	∓	∓
N.P. 16	+	+	+	+	±	±
N.P. 25	+	+	+	+	±	±
N.P. 11	+	+	+	+	±	∓

of the living 24-hour growth from one glucose ascitic agar culture, in Table III B the growth from two tubes. The tubes together with the control serum dilution were incubated at 55°C. for 2 hours and placed over night in the ice-chest. After centrifuging, the agglutination tests were made upon the homologous strain (the culture suspension of the homologous strain in A was a little less agglutinable than that used in B). Almost complete absorption occurred with four cerebro-spinal strains, including the homologous, and with one naso-pharyngeal strain; of the remaining some showed slight absorption, others none.

#### *Experiment 2.*

M. 23 serum absorbed with cerebro-spinal and naso-pharyngeal strains.

In Table IV A the method of absorption was similar to that in Experiment I. Table IV B shows the effect of absorption on two successive occasions, each time with the growth from a single glucose ascitic agar tube.

The homologous strain alone absorbed the specific agglutinin completely with a single treatment. Different degrees of absorption were shown by several naso-pharyngeal strains, complete with N.P. 8, after the second treatment.

#### *Experiment 3.*

In absorption experiments 1 and 2, where sera M. 8 and M. 23 have been treated on a single occasion with culture N.P. 11, the agglutinating capacity of these sera towards the homologous strains has

TABLE IV\*.

## A.

Absorbing strain (Control serum)	Dilutions of absorbed serum tested upon homologous strain					Titre of absorbing strain for M. 23 serum before absorption
	40	80	160	320	640	
M. 23	+	+	+	+	±	...
M. 24	+	±	trace	trace	—	500
M. 22	+	+	±	±	trace	600
M. 20	+	+	±	±	trace	800
M. 32 <sub>A</sub>	+	+	+	±	±	±
M. 33	+	+	+	+	±	±
M. 32	+	+	+	+	±	±
M. 8	+	+	+	+	±	±
M. 9	+	+	+	+	±	±
M. 16	+	+	+	+	±	—
N.P. 6	+	+	±	±	trace	800
N.P. 8	+	±	±	±	—	400
N.P. 9	+	+	±	±	±	400
N.P. 10	+	+	±	±	—	500
N.P. 11	+	+	+	±	±	400
N.P. 12	+	+	+	±	±	50
N.P. 13	+	+	+	+	±	50
N.P. 14	+	+	+	+	±	±
N.P. 3	+	+	+	+	±	±

\* In this and the following tables N.P. = non-contact naso-pharyngeal strain.

## B.

Absorbing strain (Control serum)	Dilutions of absorbed serum tested upon homologous strain					Titre of absorbing strain for M. 23 serum before absorption
	60	120	240	480	960	
N.P. 8	+	+	+	+	±	...
N.P. 10	±	±	trace	—	—	400
N.P. 6	+	±	trace	—	—	500
N.P. 7	+	+	±	trace	—	800
N.P. 24	+	+	±	±	trace	400
N.P. 9	±	±	±	trace	trace	—
N.P. 4	+	+	+	±	±	400
N.P. 17	+	+	+	+	±	—
N.P. 18	+	+	+	+	±	—
N.P. 29	+	+	+	+	±	—
N.P. 26	+	+	+	+	±	±
N.P. 16	+	+	+	+	±	±
N.P. 12	+	+	+	+	±	±

not been appreciably diminished. In the following experiment sera M. 8 and M. 23 were treated on four successive occasions with culture N.P. 11. The concentration of serum was 1 in 20, and in each case 4 c.c. were exhausted with the growth from 7 glucose agar tubes divided into four portions. After each addition of culture the tubes were incubated for 2 hours at 55° C. and centrifuged; tubes containing serum dilution alone were incubated and centrifuged at the same time. The sera were treated in identical manner with the other cultures included in the tables.

Tables V and VI show that N.P. 11 absorbs agglutinin from both sera, the absorption of M. 23 serum being the more marked. N.P. 29 and 30, both pigmented strains, the latter a laevulose fermenter, were used as controls; they have not absorbed any of the specific agglutinin. N.P. 10 has already been shown (Table IV A) capable of absorbing M. 23 agglutinin.

TABLE V.

*M. 8 serum.*

Absorbing strain (Serum control)	Dilutions of absorbed serum tested upon homologous strain				
	80	160	320	640	1280
+	+	+	+	±	—
N.P. 29	+	+	+	±	—
N.P. 30	+	+	+	±	—
N.P. 11	+	±	±	—	—

TABLE VI.

*M. 23 serum.*

Absorbing strain (Serum control)	Dilutions of absorbed serum tested upon homologous strain				
	80	160	320	640	1280
+	+	+	+	±	trace
N.P. 30	+	+	+	±	trace
N.P. 10	±	±	trace	—	—
N.P. 11	±	±	trace	—	—

*Experiment 4.*

M. 8 serum absorbed on three successive occasions with N.P. 11 culture (Table VII): 10 c.c. of 1:10 dilution of serum treated with the growth from 10 glucose agar tubes. The serum after absorption was tested as to its agglutinating capacity upon the homologous strain and upon several cerebro-spinal and naso-pharyngeal strains. The results show that N.P. 11 culture has absorbed from M. 8 serum the agglutinin that had, before the absorption, acted upon eight strains of meningococci.



TABLE VII.

Strain	50	100	200	300	400	500	600	Titre of tested strain for the unabsorbed serum
M. 8	+	+	+	±	±	±	±	400
M. 3	+	+	+	+	±	±	±	800
M. 9	-	-	-	-	-	-	-	400
M. 11	+	±	-	-	-	-	-	200
M. 15	trace	-	-	-	-	-	-	100
M. 16	trace	-	-	-	-	-	-	100
M. 14	±	-	-	-	-	-	-	100
M. 21	-	-	-	-	-	-	-	100
M. 13	-	-	-	-	-	-	-	100
M. 12	-	-	-	-	-	-	-	100
M. 17	-	-	-	-	-	-	-	50
N.P. 1	+	+	+	+	±	...	...	800
N.P. 2A	trace	-	-	-	-	...	...	100
N.P. 2	±	±	±	trace	trace	...	...	200
N.P. 11	-	...	...	...	...	...	...	50

*Experiment 5.*

M. 23 serum absorbed on three successive occasions with N.P. 11 culture and tested upon the homologous strain, in addition to several cerebro-spinal and naso-pharyngeal strains: 15 c.c. of 1:10 dilution of serum treated with the growth from 12 glucose agar tubes. The results show (Table VIII) that the agglutinins for several of the

TABLE VIII.

Strain	50	100	200	300	400	Titre of tested strain for unabsorbed serum
M. 23	+	+	±	±	trace	500
M. 18	+	+	±	±	-	1000
M. 22	-	-	-	-	-	600
M. 26	±	±	-	-	-	400
M. 28	+	±	±	trace	...	400
M. 19	+	±	±	trace	trace	1000
M. 20	+	±	trace	trace	...	800
M. 21	+	±	trace	...	...	800
M. 24	+	+	±	±	-	500
M. 29	+	±	trace	trace	...	400
M. 25	±	±	-	-	-	400
M. 30	±	±	-	-	-	400
M. 27	+	±	trace	trace	-	400
N.P. 11	-	-	-	-	-	400
N.P. 6	±	±	±	trace	-	800
N.P. 10	+	±	±	trace	-	500
N.P. 9	±	-	-	-	-	400
N.P. 8	±	±	trace	-	-	400
N.P. 7	+	±	±	trace	-	400

cerebro-spinal meningococcal strains are reduced, and in one case, M. 22, completely absorbed.

*Experiment 6.*

Absorption experiment with M. 9 serum (Table IX). In each case 1·5 c.c. of 1 : 10 dilution of serum were treated with the growth from a single glucose ascitic agar tube.

M. 8 and M. 9 have absorbed almost completely the specific agglutinin from M. 9 serum. In Experiment 1 M. 9 was shown to absorb the specific agglutinin from M. 8 serum.

TABLE IX.

Absorbing strain (Control serum)	Dilutions of absorbed serum tested upon homologous strain				
	80	160	320	640	1280
(Control serum)	+	+	+	±	trace
M. 8	trace	—	—	—	—
M. 9	trace	—	—	—	—
M. 16	+	+	+	trace	—
M. 11	+	+	+	trace	—
M. 13	+	+	+	trace	—
M. 14	+	+	+	trace	—
M. 17	+	+	+	trace	—
M. 15	+	+	±	∓	—
M. 12	+	+	±	∓	—
N.P. 11	+	+	+	∓	—

ANALYSIS OF ABSORPTION EXPERIMENTS.

Absorption experiments 1 and 2 demonstrate that strains which on the score of agglutinogenic capacities or agglutinability might be regarded as belonging to the same group do not necessarily coincide in absorptive capacities. In Experiment 1 there is a correspondence between agglutinability and absorptive capacity, but not in Experiment 2.

Experiment 3 shows that a naso-pharyngeal strain has absorbed specific agglutinin from sera of both groups of cerebro-spinal meningococci and that absorption was more marked with that serum which agglutinated the absorbing strain.

Experiment 4 shows that a naso-pharyngeal strain has removed from a cerebro-spinal meningococcus serum a certain amount of the agglutinin for the strain homologous to that serum, but has removed

all the agglutinin for a second strain, which (Experiments 1 and 6) has been shown to be identical with the homologous strain.

Experiment 5 shows that a naso-pharyngeal strain has removed from a meningococcus serum, to an unequal extent, the agglutinin for other strains belonging to the same group.

Experiment 6 demonstrates equal absorptive capacity in M. 8 and M. 9, members of the same group.

#### SUMMARY OF RESULTS.

Simple agglutination tests divided into two main groups a series of 34 meningococci obtained from the cerebro-spinal fluid of cases of meningitis during the recent epidemic.

Certain of these strains of meningococci, which were either not agglutinated or only slightly by any of the sera employed, could be placed in one or other of the groups by the demonstration of their agglutininogenic capacity.

Certain individual strains in each of the groups were agglutinated to a less degree than the homologous strain, and certain strains were agglutinated by sera of both groups.

For further evidence as to specific relationship resort was made to agglutinin absorption experiments.

While the question, as to whether meningococci can be divided into independent groups by means of agglutinin absorption tests, must, at this stage of the investigation, remain open, the experiments detailed above indicate that variations in absorptive capacity between individual strains of meningococci are analogous to, though not in actual correspondence with, variations in agglutinability.

The non-contact naso-pharyngeal strains, culturally identical with meningococci, exhibited in relation to monovalent agglutinating sera prepared with cerebro-spinal meningococci, the same tendency to grouping as the cerebro-spinal strains and similar variations in agglutinability. The 28 non-contact strains, which have been investigated serologically, reacted to the following extent with one or other of the above-mentioned meningococcus immune sera:—5 showed complete agglutination in 1:400 or over, 10 in 1:200 or over, 6 in 1:100; 7 were not completely agglutinated in dilutions higher than 1:50. The first-mentioned 5 absorbed, from the respective agglutinating sera, the agglutinins for the homologous strains. From one of these 5, N.P. 10,

a serum was prepared which was found to agglutinate strains in Group II. Of the 16 strains which agglutinated with meningococcus sera between 1 : 100 and 1 : 400, a few were tested as to their absorptive capacity in relation to two sera and, as will be seen from the absorption tables, showed evidence of agglutinin absorption. The absorption, though slight in amount, was equal to that occurring with the same sera treated with certain cerebro-spinal strains. The remaining 7 strains, which were agglutinated feebly by the meningococcus sera employed, are being subjected to further investigation (1) as to their agglutinability in relation to other cerebro-spinal meningococcus sera, (2) as to their capacity for producing agglutinating sera for cerebro-spinal meningococci. Taken as a whole, the serological results afford indication of a division of meningococci into two groups with some overlapping of each.

The theoretical explanation may be that the antigenic substance of the meningococcus contains one or other of two specific components, A and B, and sometimes contains both components, one of the two then being present in greater amount than the other. Consequently some strains produce sera with agglutinins of the A class alone; others create agglutinins of the B class alone; others produce both A and B agglutinins, with preponderance in some cases of A and in other cases of B. As regards agglutinability, again, some strains are capable of combining with A alone, others with B alone, and others with both A and B, but to a greater degree with the one than with the other.

Comparing the capacity of an individual strain for producing agglutinin with its capacity for combining with agglutinin, I find that in some cases these two capacities appear to coincide. But this is not a general rule. For example, a strain may have limited capacity for combining with agglutinin, but much greater capacity for producing agglutinin; again, it may combine with A alone, or mainly with A, but produce agglutinins in which B preponderates over A.

Absorption experiments, again, show that whilst there is sometimes a correspondence between capacity to absorb and capacity to create, or combine with, specific agglutinin, this correspondence is not a general rule.

These last two considerations show that the characteristics of different strains of meningococci, while affording a basis for division into groups, are closely inter-related, and, in fact, are connected by inseparable links, which appear to make it impossible to effect a definite cleavage between the one group and the other.



The above observations have been suggested by the results so far obtained, but further work on this subject is in progress, with special reference to the identification of the meningococcus in the naso-pharynx.

#### CONCLUSION.

All strains of Gram-negative cocci, obtained from the naso-pharynx, identical microscopically, culturally and in fermentation tests with meningococci, must, in default of a specific test for virulence and until the serological relationships have provided definite evidence to the contrary, be considered to be meningococci.

# A STUDY OF MENINGOCOCCI OCCURRING IN THE SPINAL FLUID AND OF SIMILAR ORGANISMS IN THE NASO-PHARYNX.<sup>1</sup>

By W. M. SCOTT, M.D.

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## INTRODUCTION.

THE work has been done in the Local Government Board's Pathological Laboratory, and during its course has had the benefit of much advice and criticism from Drs Eastwood and Griffith, the Board's pathologists.

The task proposed was threefold, (1) to determine the period during which cases of cerebro-spinal fever remain infective, *i.e.*, for how long during convalescence meningococci can be cultivated from the naso-pharynx; (2) to establish if possible the specific identity of meningococci isolated from the cerebro-spinal fluid with those got from the naso-pharynx of the same case; (3) while these pathological throats were being studied, and providing presumably pathogenic meningococci, to search among the flora of the normal, or at least "non-contact" naso-pharynx, for micro-organisms liable to be mistaken for pathogenic meningococci or actually indistinguishable from these. Some opportunity was also afforded for observing whether meningococci were present in the blood stream during the acute stage of cerebro-spinal fever.

The case material was provided by Dr Foord Caiger, of the Metropolitan Asylums Board South-Western Fever Hospital, to whom I wish to express my very great indebtedness for the large facilities he granted for visiting and examining the cerebro-spinal fever cases under his care.

Dr A. L. Baly, the Medical Superintendent of the Lambeth Infirmary, was kind enough to allow me to take cultures from the naso-pharynx of "non-contacts" attending his out-patient department.

I have pleasure also in thanking Dr Arkwright, of the Lister Institute, who gave me cultures of 25 strains of meningococci which he had isolated from the spinal fluid of cases of cerebro-spinal fever during the recent epidemic.

## A. INVESTIGATION OF CEREBRO-SPINAL FEVER CASES.

*Technique of Culture.*

*Naso-pharyngeal Cultures.* The examination of the naso-pharynx in all cases was performed personally by abstracting a small portion of mucus from the posterior nares by means of a swab attached to the end of a bent rod, taking care to avoid contamination by the fauces or buccal mucosa. In the case of patients unable to sit up I found it difficult to avoid such contamination, and many of the "negative"

swabs recorded during the acute stages of the disease were due to overgrowth of the plates by contaminating organisms.

The mucus abstracted was deposited immediately on freshly poured plates: these were conveyed without delay to the laboratory (usually within one hour), and there the mucus was rubbed over the plate with a right-angled glass rod, this rod being then used for inoculating a second plate of the same medium.

Plates were examined after 24 hours' incubation at 37° C. and again after 48 hours. Suspected colonies were examined first microscopically; when typical micrococci were found a little of the colony was emulsified in sterile saline and used for inoculating tubes of solidified egg, ordinary nutrient agar, ascitic agar, and ascitic agar containing one of each of the following sugars—glucose, maltose, saccharose, and levulose. Strains corresponding culturally with the meningococcus were kept on egg in sealed tubes at 37° C. On this medium I found that they maintained their vitality for at least four months without sub-culture, though routine sub-culture was performed on egg at intervals of one or two months.

Cultures from meningeal fluid were isolated in similar fashion from plates inoculated with the deposit from the centrifuged fluid.

*Media Employed for Primary Plates.* Kutscher's serum-agar was always employed. It was prepared from fresh human placenta 500 grams to the litre, a boiled extract being made as with meat after mincing; to this nutrose 2 %, glucose 1 %, peptone (Chapoteaut) 1.5 %, and agar 2.5 % were added, the reaction being brought to + 8 (Eyre) after steaming; this was stored solid in bottles containing 75 c.c., which for use were melted and brought to 50° C.; 25 c.c. of sterile (filtered) ox serum were then added and the mixture poured at once. Comparison with similar agar lacking the placental extract showed that on primary cultivation the meningococcus-like organisms on Kutscher's medium grow more rapidly and produce larger colonies before growth stops.

The medium used for fermentation tests was litmus ascitic agar brought to + 5 (Eyre), the different sugars being added in sterile solution to make 1 % just before sloping.

#### *Microscopical and Cultural Characters.*

*Microscopically* meningococci are micrococci varying considerably in size in different strains in primary colonies and showing much variation in size in individuals of the same colony: giant forms are usually present.



The *arrangement* is typically diplococcal, usually with distinct flattening of adjacent poles: tetrads are common but not invariably present. The presence of chain formation or pronounced staphylococcal groups rules out a colony as certainly not meningococcal.

*Staining* in young cultures is distinctly uniform with the exception of the giant forms which often overstain. All individuals are definitely Gram-negative [carbol-gentian-violet  $\frac{1}{2}$  minute, Gram's iodine  $\frac{1}{2}$  minute, absolute alcohol  $\frac{1}{2}$  minute].

*Colonies* on Kutscher's agar are highly characteristic. After 24 hours they appear perfectly circular, slightly convex, with a sharp margin, and measure from 1 to 2 mm. across. The colour is pearl-grey, and when viewed by transmitted light they may show slight iridescence: this latter appearance, however, is much more common with colonies from cerebro-spinal fluid than with throat colonies, a fact which may depend on the material inoculated and on the presence or absence of contaminating organisms. The most typical feature, and that on which most reliance was placed, is the fine granularity when viewed under a  $\times 8$  lens on the stage of a dissecting microscope and illuminated strongly but obliquely by tilting the mirror so that no direct rays reach the lens.

The consistence is also characteristic: the growth adheres well to the platinum needle without being tenacious; it is not watery nor does the colony break into fragments on touching. The colony is moist and emulsifies readily in water.

After 48 hours the colonies may reach 3 to 4 mm. in diameter but remain perfectly circular: they are less transparent in the centre, but do not become coarsely granular. Some strains acquire a rather characteristic ringed appearance after 24 hours, due apparently to a thinner zone of the colony between the centre and the outer part; these were mostly found to fall into a group, the "Clayton" group, which was distinguishable, as will be seen later, by fermentation and agglutination tests. Some members of this group, however, did not show this ringed appearance. There is never any *pigment* in primary colonies, but sub-cultures on egg usually show a faint orange-pink colour in the growth collected on a loop. This may even reach a distinct orange or buff colour with certain undoubted strains. Lemon-yellow pigment always excludes a colony; some primary colonies are perfectly colourless, but on sub-culture develop this pigment and can then be excluded on this as well as on other grounds.

Growth on ordinary agar slopes at 37° C., inoculated with an emulsion from the primary colony, never occurred with any strain

which passed the further tests. A certain number of pigmented organisms, however, also failed to grow on ordinary agar.

Growth at 22° C., on tubes similarly inoculated, depends on the medium employed: on ascitic agar it rarely, if ever, occurs, but on Kutscher slopes, as also on egg, definite colonies appear quite frequently in two to four days; on the latter media growth is usually abundant if the inoculation is made with a considerable mass of the colony.

#### *Fermentation Tests.*

The characteristic behaviour is fermentation of glucose and maltose only, but it has not been thought justifiable to reject strains which, inoculated from the primary colony, fermented only one of these two substances. Such single-sugar fermenters on later sub-culture ferment both sugars as a rule, though one may be only slightly attacked. One cerebro-spinal fluid strain, however, fermented no sugar when first isolated, and later fermented glucose only. All the yellow-pigment formers which were isolated fermented levulose in addition to these two sugars, and most of them also saccharose.

*Differences in degree of fermentative activity and comparison with agglutination.* Of the cerebro-spinal fluid strains sub-cultured from primary colonies five fermented glucose more strongly than maltose, seven *vice versâ*, while two fermented them equally. Of the cerebro-spinal fluid strains isolated by Dr Arkwright, I found that eight fermented glucose more strongly than maltose, eleven *vice versâ* and six equally. Of the strains isolated from the naso-pharynx of convalescent patients nineteen fermented glucose more strongly than maltose, ten *vice versâ* and ten equally.

The glucose-preferring strains showed often but not always the "ringed" type of colonies; they were further separated by the *agglutination* tests which follow and form a fairly definite group, the "Clayton" group (*v. infra*). Most of the maltose-preferring strains and most of the equal-fermenters fell into the "Boscombe" agglutination-group. Exceptions occurred however: one glucose-fermenting strain fell into the Boscombe group, and several equal-fermenters were placed by agglutination in or closely related to the Clayton group. The question of grouping will be discussed after describing the agglutination reactions, but it may be stated that on the strength of observation of the relative activity with which these two sugars were fermented, I could generally predict with which serum the strain would agglutinate.

## AGGLUTINATION TESTS.

*Technique of Preparation of Sera.*

Monovalent sera alone have been used, and rabbits have been successfully immunised with four *strains*—Boscombe, Clayton, Chandler and Smith. Boscombe and Clayton were strains found in almost pure culture in the *naso-pharynx* of cases of cerebro-spinal fever early in convalescence (1 week and 4 weeks respectively from commencement of the disease). They were chosen as representing the best marked examples in my possession of predominating fermentation of maltose and glucose respectively, the other sugar in each case being only slightly attacked. Chandler and Smith were selected later on the ground of feeble agglutination with Boscombe and none with Clayton serum. They were isolated from the *cerebro-spinal fluid*, Chandler by Dr Arkwright, Smith by myself. Both fermented maltose and glucose equally strongly.

In the *preparation* of the sera two methods have been used. For Boscombe and Clayton sera prolonged immunisation was employed, but the agglutinating titre was not raised much higher in the succeeding three months of treatment than it appeared ten days after the second injection. In the case of Chandler and Smith sera of equal potency to the above were obtained in a fortnight by giving two maximum intravenous doses at an interval of three days. Ten days later a satisfactory serum was obtained from four of the six animals; two died (one with each strain) 24 hours after the second dose.

The *antigens* in the case of Boscombe and Clayton were living cultures on solidified egg, the dose being raised from  $\frac{1}{2}$  a culture to  $\frac{2}{3}$ , and finally to a whole culture; the injections were made intravenously at intervals of one week. The animals all lost weight during the first two months, but were regaining it during the later stages of immunisation. They were young rabbits of 1500 grams to 1800 grams. In the case of Chandler and Smith whole living cultures on Kutscher's slopes were injected intravenously on each occasion.

The titre of all these sera may be stated as complete agglutination of the homologous strain in a dilution of 1 in 600; well-marked agglutination with some remaining turbidity took place at 1 in 800; higher dilutions were not systematically employed.

*Failures.* One rabbit of three immunised with Clayton never produced a serum agglutinating completely at a higher dilution than

1 in 200. With two other strains, one from the throat and one from the lumbar fluid, all the rabbits employed (two and three respectively) failed to produce sera agglutinating completely higher than 1 in 200. These sera have not been used for systematic tests pending repetition of the attempt to produce with the strains sera of higher titre. The failure may depend on poor agglutinogenic properties in the *antigens*, but it must be taken into account that rabbits vary much in their response to immunisation.

#### *Technique of agglutination tests.*

The macroscopic method was used throughout and the mixtures were incubated for 24 hours at 55° C.

The micrococcal emulsions were made from glucose ascitic agar cultures of 24 hours' incubation, sown from 24 hours' egg cultures; the growth was washed off with saline, the emulsions thoroughly shaken, allowed to stand for 4 hours and then pipetted off so as to avoid coarse flakes of growth. Such emulsions remain in suspension for 2 to 4 days without producing visible deposit.

Newly-made emulsions, unheated, were used for all tests. All were brought to a standard opacity by comparison with a standard barium sulphate suspension corresponding in opacity to an emulsion containing 10 mg. moist growth per c.c.

The mixtures were put up in Durham's tubes calibrated so that equal portions were marked off, each containing about 0.3 c.c.; the diluted serum was first put in, then the emulsion run in quickly so as to insure mixture. A control of normal rabbits' serum diluted 1 in 100 was put up on each occasion with each emulsion.

#### *Variations in agglutination.*

Four to five agglutination tests have been made with each strain at intervals of several weeks during which the strains remained in cultivation.

The great majority of the strains kept were very stable throughout both as regards the dilution of a particular serum required to produce complete agglutination and as regards the specificity, *i.e.*, the presence or absence of agglutination with different sera. This stability was no doubt favoured by the fact that the stock was maintained on solidified egg at 37° C., and the sub-cultures employed for agglutination tests,



with three exceptions, had not more than three or four sub-cultures intervening between them and the original colony.

None of the strains isolated from the lumbar fluid varied to a greater degree than might depend on small errors in the technique of the agglutination test, with the exception that with one strain, A 2, a gradual increase in agglutinability took place; originally giving only slight clumping with 1-100 Boscombe serum, it increased to complete agglutination at 1-100 and definite clumping at 1-200.

On the other hand one of the South-Western Hospital naso-pharynx strains, T. 1a, became suddenly inagglutinable or rather incapable of complete agglutination since clumps appeared at as high a dilution as before, but turbidity persisted in the strongest serum. An emulsion prepared from a 24 hours' growth sown from an old stock culture, two "generations" instead of six from the original colony, agglutinated to the original titre. The same phenomenon appeared again later with this strain, but was not so well marked. Both A. 2 and T. 1a were strains sub-cultured more frequently than the others owing to the fact that they were being used for immunisation, and this may account for the variation.

Four strains developed "auto-agglutinating" properties. In one case by returning to an old stock the original characters were recovered; another strain died out in spite of all efforts to revive it. The two others lost the auto-agglutinating property on further sub-cultivation. All four were throat strains and the appearance of auto-agglutination coincided with evident diminution in the vigour of growth.

#### *Agglutination of spinal strains.*

Tables are subjoined recording the results of agglutination tests with 14 strains from the South-Western Fever Hospital, and 25 isolated by Dr Arkwright. It will be observed that with each serum the maximum dilution is given which produced complete agglutination, *i.e.*, perfectly clear supernatant fluid above the mass of agglutinated cocci; with each serum the homologous strain showed this at 1 in 600. With most strains well-marked clumping, but with some remaining turbidity of the liquid, took place at considerably higher dilutions, this being represented in reading results with the mark ++. Dilutions higher than 1-800 were not used for systematic tests as it was considered that the complete agglutination point was the most satisfactory one for comparison. The ++ point is of some value in comparing poor agglutinators where no complete agglutination occurred.

TABLE I.

*Agglutination Reactions of Cerebro-Spinal Fluid Strains  
from South-Western Fever Hospital.*

Strain	Serum Boscombe		Serum Clayton		Serum Chandler		Serum Smith	
	Highest dilution with complete agglutination	Highest dilution with agglutination ++	Highest dilution with complete agglutination	Highest dilution with agglutination ++	Highest dilution with complete agglutination	Highest dilution with agglutination ++	Highest dilution with complete agglutination	Highest dilution with agglutination ++
C.S. 1	600	800	—	—	100	200	600	800
„ 2	600	800	—	—	100	200	600	800
„ 3	600	800	—	—	200	200	600	800
„ 4	400	800	—	—	200	400	200	400
„ 5	400	600	—	—	—	100	400	800
„ 6	400	800	—	—	—	200	200	800
„ 7	600	800	—	50	200	400	600	800
„ 8	100	400	—	50	100	200	600	800
„ 9	—	100	400	600	—	—	50	100
„ 10	—	—	800	800	—	—	—	100
„ 11	—	—	400	400	—	—	—	—
„ 12	—	—	400	600	—	—	—	—
„ 13	—	—	400	600	—	—	—	—
„ 14	—	—	200	400	—	—	—	—
Blood 1	—	—	400	400	—	—	—	—

The table shows that strains C.S. 1 to 7 agglutinate strongly with the sera Boscombe and Smith, moderately with serum Chandler and are negative with serum Clayton except for slight agglutination at 1-50 with C.S. 7.

Strain 1 is from the cerebro-spinal fluid of Boscombe, whose nasopharynx strain was used for preparing the serum of that name.

Strain 8 is Smith and agglutinates accordingly to the full titre with its own serum; it responds rather feebly to Boscombe and Chandler and is almost but not quite negative to Clayton.

Strain 9 agglutinates strongly with Clayton, weakly with Smith, is negative to Chandler and almost but not quite so to Boscombe. Strains 10 to 14 and the blood strain, which was got from the same patient as strain 11, respond strongly to Clayton and are negative to the other three sera with the exception of 10 which reacts very slightly to Smith.

So far, then, a rough but fairly satisfactory grouping into two sets is evident with these strains of cerebro-spinal origin; Boscombe, Chandler and Smith sera pick out eight of the fifteen strains and reject almost but not quite entirely the remaining seven which respond instead to the Clayton serum.

On applying this grouping, however, to a further set of cerebro-spinal strains, those supplied by Dr Arkwright of the Lister Institute, evidence appears of additional affinities which bring into relationship strains which, on the strength of agglutination with Boscombe and Clayton sera, appear to be sharply distinguishable.

TABLE II.

*Comparison with strains supplied by Dr Arkwright.  
Agglutination Reactions of Dr Arkwright's Cerebro-Spinal Strains.*

Strain	Boscombe		Clayton		Chandler		Smith	
	Highest complete agglutination	Highest ++ agglutination	Highest complete	Highest ++	Highest complete	Highest ++	Highest complete	Highest ++
A. 1	400	800	—	—	200	400	200	400
A. 2	100	200	—	—	600	800	200	800
A. 3	600	800	—	—	—	200	800	800
A. 4	600	800	—	—	100	400	400	800
A. 5	600	800	—	—	100	200	400	800
A. 6	800	800	—	—	200	800	400	800
A. 7	200	800	—	—	100	200	800	800
A. 8	600	800	—	—	200	600	400	800
A. 9	400	800	—	—	100	200	400	800
A. 10	200	800	—	—	600	800	200	400
A. 11	600	800	—	—	100	200	400	800
A. 12	600	800	—	—	100	200	400	800
A. 13	200	400	—	—	100	400	400	800
A. 14	400	800	—	—	—	200	400	800
A. 15	600	800	—	100	100	200	400	800
A. 16	200	800	—	—	—	200	200	400
A. 17	400	800	—	—	100	200	800	800
A. 18	400	800	—	—	—	100	400	800
A. 19	—	—	600	800	—	—	—	—
A. 20	—	—	400	800	—	—	—	—
A. 21	—	—	600	800	100	200	—	—
A. 22	—	—	400	800	400	600	—	—
A. 23	—	100	200	400	200	400	—	—
A. 24	—	—	200	800	100	200	400	800
A. 25	—	—	400	800	100	200	400	800

Here again the Boscombe and Clayton sera make a fairly sharp division, there being eighteen responding definitely to Boscombe and not appreciably to Clayton serum, while with the remaining seven the response is exactly reversed.

But among those agglutinated by Boscombe serum, one, A. 2, agglutinates weakly, the complete reaction not extending above 1-100. This is Chandler, which provides the serum of the name, and this serum

picks out along with fourteen of the Boscombe type four of the Clayton group which it agglutinates on the average quite as strongly.

Further the Smith serum, while it agglutinates all the Boscombe strains fairly strongly, also agglutinates two which are definitely of the Clayton type.

There is evidence thus that it is easy to erect groups among a collection of meningococcal strains on the basis of agglutination with single-strain sera, and it is interesting to note that the fairly definite grouping brought out by Boscombe and Clayton sera coincides very closely with the grouping by means of the fermentation tests referred to above. On the other hand, members of these groups have affinities unrevealed by the particular pair of sera chosen for grouping. In the table above it is evident that the gap between the well-defined Boscombe and Clayton groups is bridged by the Chandler agglutinated strains and by those agglutinated by serum Smith.

It may eventually be possible to correlate by agglutination tests all the different strains of meningococci which occur in nature and to align them in a series. In such a correlated series the Boscombe and Clayton cultures would occupy opposite ends and be connected with each other by a great number of gradually differing but closely related strains. The central members of the alignment would differ equally widely from both Clayton and Boscombe, while the Boscombe and Clayton type characters would increasingly predominate in the strains towards one or other end.

*Agglutination Reactions of Meningococcus-like Organisms in  
the Naso-pharynx of Convalescents.*

Table III expresses the behaviour as regards agglutination with the same four sera of strains of micrococci which produced characteristic colonies on plates inoculated from the naso-pharynx of convalescents and which microscopically and culturally were indistinguishable from the meningococcus. No strain which passed the morphological and cultural tests failed to give complete agglutination with one or more sera diluted 1-100.

Each arabic numeral represents an individual patient while the letters attached indicate that the strain was isolated from the same patient on different occasions, the dates of which from the time of admission to hospital are given in the second column. Further, an additional column not included in previous tables is devoted to recording



TABLE III.

*Naso-pharyngeal Strains from South-Western Fever Hospital.**Cases of Cerebro-spinal Fever and Contacts.*

No. of case	Period after admission at which strain was isolated	Boscombe serum			Clayton serum			Chandler serum			Smith serum		
		Highest complete	+	Agglutination at 1-100	Highest complete	+	Agglutination at 1-100	Highest complete	+	Agglutination at 1-100	Highest complete	+	Agglutination at 1-100
T. 1a	6 days	600	800	c	-	-	+	100	200	c	600	800	c
T. 1b	2 months	-	100	++	-	100	++	200	600	c	-	100	+++
T. 1c	10 weeks	-	100	++	100	200	c	200	400	c	100	200	c
T. 1d	3 months	-	100	++	-	200	+++	-	100	++	200	800	c
T. 2a	3 weeks	400	600	c	-	-	trace	-	-	+	-	100	++
T. 2b	5 weeks	400	600	c	-	-	+	-	-	+	-	200	+++
T. 2c	7 weeks	400	600	c	-	100	++	-	200	+++	400	800	c
T. 3a	10 days	200	400	c	-	-	-	100	...	c	600	800	c
T. 3b	3 weeks	400	600	c	-	-	-	...	...	...	...	...	...
T. 4a	6 days	600	800	c	-	-	-	-	100	++	400	800	c
T. 4b	2 weeks	-	-	+	400	800	c	...	...	...	...	...	...
T. 4c	4 weeks	-	-	+	400	800	c	...	...	...	...	...	...
T. 4d	7 weeks	-	-	-	600	800	c	-	100	++	-	200	+++
T. 4e	8 weeks	-	-	-	400	800	c	...	...	...	...	...	...
T. 5	5 days	600	800	c	-	-	-	-	-	+	100	400	c
T. 6a	9 days	400	600	c	-	100	++	-	200	+++	400	800	c
T. 6b	40 days	400	600	c	-	-	+	-	200	+++	400	800	c
T. 7	2 days	200	800	c	-	-	+	100	200	c	600	800	c
T. 8	1 day	-	-	trace	200	400	c	-	100	++	100	200	c
T. 9	Contact, 3 weeks	-	-	-	400	800	c	-	-	trace	-	-	trace
T. 10	3 weeks	-	100	++	200	600	c	-	100	+++	-	100	+++
T. 11a	6 weeks	-	-	trace	100	200	c	-	-	-	-	-	-
T. 11b	7 weeks	-	-	-	200	400	c	-	-	trace	-	-	+
T. 12a	4 days	-	-	+	600	800	c	-	100	++	-	200	+++
T. 12b	3 weeks	-	-	-	400	800	c	-	-	+	-	100	++
T. 13a	4 weeks	-	-	trace	600	800	c	-	-	-	-	-	-
T. 13b	5 weeks	-	-	-	600	800	c	-	-	-	-	-	-
T. 13c	6 weeks	-	-	+	600	800	c	-	-	+	-	-	+
T. 13d	7 weeks	-	-	-	600	800	c	-	-	+	-	-	+
T. 13e	8 weeks	-	-	+	600	800	c	-	-	-	-	-	+
T. 13f	9 weeks	-	-	-	600	800	c	-	-	-	-	-	+
T. 14	3 weeks	-	-	+	200	400	c	-	-	-	-	-	-
T. 15	10 days	-	-	-	100	200	c	-	-	-	-	-	-
T. 16	4 weeks	100	200	c	-	-	+	-	-	+	-	-	+
T. 17a	Contact, 2 weeks	-	-	+	200	400	c	-	-	+	-	-	+
T. 17b	Contact, 3 weeks	-	-	+	200	400	c	-	-	+	-	100	++
T. 18	Contact, 3 weeks	-	-	-	100	200	c	-	-	+	-	100	++
T. 19	Contact, 2 months	-	-	trace	400	600	c	-	-	-	-	100	++

with each serum its agglutinating effect when diluted 1 in 100. The sign "+++" indicates that agglutination was almost but not quite complete, faint turbidity persisting, while "+" indicates clumping present but not well-marked. The "++" mark indicates, as before, well-marked agglutination with a good deal of turbidity persisting.

It will be seen that 12 of the 38 strains agglutinate most strongly with Boscombe serum, while 23 strains agglutinate most strongly with the Clayton serum; two strains agglutinate most strongly with Chandler and one with Smith.

Of those agglutinating completely with Boscombe one does not do so higher than at 1-100, but all but two of the others reach 1-400, while ten of those agglutinated completely by Clayton serum do not exceed the level of 1-200. Four of these last were from contacts with no meningeal symptoms who presented doubtful if any clinical evidence of naso-pharynx infection. The possibility will be shown later, when discussing non-contacts, that some of these strains were not abnormal inhabitants of the naso-pharynx.

*Persistence of Meningococci in the naso-pharynx of cases  
of Cerebro-spinal Fever.*

In 16 cases regular examination of the naso-pharynx was possible. In eight of these, patients 1, 2, 4, 10, 11, 12, 13, 16, meningococcus-like organisms were got up to the end of treatment in hospital and were still presumably present on discharge, in one case after over 3 months, after 9 weeks in another, and 7 weeks in 3 others; the periods at which meningococci were found are shown in Table III.

Thus in 50 % of cases complete convalescence occurred before the naso-pharynx was free; the maximum period of persistence of meningococci was not determined but evidently exceeded 3 months.

In five cases negative results were obtained on all (at least two) successive examinations after positive results had been established; the last positive result was on the fifth day in one case and at various periods up to five weeks in the others: *vide* Table III, patients 3, 5, 6, 7 and 15. In the three remaining, undoubted cases of disease, no meningococcus-like organism was found in the naso-pharynx either during the acute stage or at any of five weekly examinations thereafter.

Cases 8, 9 and 14 in Table III presented doubtful symptoms of cerebro-spinal fever, but were not established as cases of the disease, spinal puncture not having been performed.

Cases 17 and 18 were contacts of the disease, but presented indefinite symptoms only and were not tapped, while case 19 was an enteric fever patient nursed in the same ward as the cerebro-spinal fever cases.

In addition, 23 patients from the cerebro-spinal fever wards were swabbed once with negative results; 10 of these were in advanced convalescence from the disease when first seen and 13 were enteric fever patients. These were the first swabs I took, and the negative results may in part be ascribed to lack of experience in examining plates from the naso-pharynx; the smaller colonies which later experience showed to be meningococcal might have been overlooked.

#### *Meningococci in the Blood Stream.*

Three attempts at cultivation of meningococci from the blood were made in three acute cases; two of these failed in spite of having incubated 9 c.c. of blood in 200 c.c. of serum broth. From the third, the most acute and eventually a fatal case, cultures were easily got by direct inoculation of a few drops of blood on slopes of Kutscher's agar. The strain thus isolated agreed in all respects with that obtained from the spinal fluid of the same case. Microscopical examination of the blood in this and five other acute cases was entirely negative as regards the appearance of diplococci within or without cells.

#### *Comparison of Strains cultivated from the Spinal Fluid and the Naso-pharynx of the same patient.*

This was possible in seven cases; the spinal strains being, in Table I, strains C.S. 1, 2, 4, 5, 7, 8, and 10, while the naso-pharynx strains corresponding are, in order, T. 1 (a, b, c and d), T. 2 (a, b and c), T. 3 (a and b), T. 4 (a, b, c, d, and e), T. 6 (a and b), T. 15, and T. 12 (a and b).

It will be seen that in all but the pair C.S. 7 and T. 15 a strain was isolated from the naso-pharynx corresponding closely in specific agglutination with that got from the spinal fluid. In four cases no other specific type than that found in the spinal fluid was cultivated from the throat.

In two cases, however, the pairs C.S. 1 with T. 1 and C.S. 5 with T. 4, only the first positive swab furnished a strain agglutinating like the spinal strain.

T. 1 a, like C.S. 1, was a strongly agglutinated "Boscombe" strain; T. 1 b, c and d were very feebly agglutinated by "Boscombe" serum,

but the first two agglutinated fairly well with "Chandler" serum and the last with "Smith" serum.

T. 4 a, like C.S. 5, was similarly of the Boscombe type, but T. 4 b, c, d, and e were almost pure Clayton strains agglutinating almost to the full titre of this serum and feebly or not at all with all other sera.

The question is raised by these results whether modification may go on in the naso-pharynx so that one type changes into the other. It is noteworthy that the plates from which these strains were obtained showed almost pure cultures of colonies indistinguishable from those picked for isolation of the strains.

An alternative hypothesis is that the later swabs were furnishing cultures of another, perhaps a normal, inhabitant of the naso-pharynx which had been swamped by the infecting strain at the time of the first examination.

The strain T. 15 which is also different from its spinal strain C.S. 8 raises the same question, but in this case the homologous strain was not recovered from the naso-pharynx, only the variant.

#### B. INVESTIGATION OF NON-CONTACTS.

##### *Meningococcus-like Organisms in the Naso-pharynx of Non-Contacts.*

The observations just discussed lead up to the question whether organisms resembling meningococci may be normal inhabitants of the naso-pharynx.

Among the Lambeth Infirmary out-patients from June 1st to July 15th, 1915, 150 swabs were taken, 38 from males, 112 from females; 12 of these were repeat swabs, 11 being seconds and 1 third. Of a total of 138 individuals 19 ( $13\frac{1}{2}\%$ ) were of 14 years or under, while 66 (47·8 %) were of 50 or over.

Twenty-six were normal individuals, the rest suffered from various ailments—chronic coughs, rheumatism, "bad legs," etc. No connection with cases of cerebro-spinal fever could be discovered with any.

Thirty-five swabs yielded colonies *culturally* and *microscopically* indistinguishable from the meningococcus. Of these, however, two were repeats reducing the percentage of individuals with such suspicious organisms to 24 %. Of these, moreover, three eventually developed abnormalities necessitating their exclusion, so that the final percentage



of "positives" on the strength of microscopical and cultural characters was 22 %.

The colonies were noted as being in "almost pure culture" in 2 cases, "numerous" in 15 cases, "few" in 8 cases, while in 10 cases a single suspicious colony was found on the plates.

#### *Cultural Characters and Fermentation Tests.*

Colonies selected were those showing sharply defined round outline, slightly raised towards centre, pearly grey in colour, translucent and with characteristic fine granularity when illuminated obliquely from below under  $8 \times$  lens.

With few exceptions on plates of 24 hours they were *smaller* and *slightly more opaque* than the meningococcus colonies isolated from the naso-pharynx of convalescent C.S.F. patients and in only two instances presented the bluish tint by transmitted light characteristic of colonies grown from the lumbar fluid. After growing for 48 hours the differences above noted were less marked, though the opacity was still rather greater in most cases.

A good many other colonies were sub-cultured as being doubtful, but not definitely distinguishable; these fermented other sugars (levulose and saccharose), grew on first sub-culture on ordinary nutrient agar, showed good growth on ascitic agar at  $22^{\circ}$  C. in two days, and were hence not further studied.

Of the 35 strains kept for further study, 2 were lost before all the tests were complete, while 2 began to develop traces of pigment on successive sub-cultures and were then found to ferment levulose in addition to glucose and maltose. A fifth without developing colour was found later to ferment levulose. The last three were tested along with the "normal" strains as regards agglutinating properties. They were entirely negative with all sera used.

Slopes of litmus ascitic agar containing the various sugars in 1 % strength were inoculated direct from suspected colonies. The results were as follows: of the 35 strains kept, 12 fermented glucose more strongly than maltose, 5 fermented maltose more than glucose, while 16 were apparently equal, and 2 fermented glucose only. Saccharose, levulose and galactose were not fermented by any. On repeating the fermentation tests after five to six months' sub-culture on egg the following results were got:—(1) 3 strains now fermented levulose, 2 by this time being known as pigment-producers (yellow), the third not

pigmented: (2) no strain which formerly preferred glucose to maltose now preferred maltose to glucose and *vice versa*, i.e., there were no reversed activities; (3) none of the previously equal-fermenters now preferred glucose; (4) but 10 of these equal-fermenters now preferred maltose; (5) 3 of those formerly preferring glucose now fermented maltose equally well; (6) 2 of those preferring maltose but also fermenting glucose now fermented maltose only. There was evidently a general increase in the maltose fermenting power as compared with glucose. The medium employed at the second examination differed in the ascitic fluid being of a different sample, but was in reaction as nearly as possible the same, + 5.

#### *Agglutination Reactions.*

The thirty surviving strains were tested as regards agglutination with the same four sera as in the previous tests and the results are shown in Table IV (p. 481).

The symbol "+" signifies definite but slight agglutination; the symbol "++" means well-marked but incomplete agglutination, while "+++" means agglutination almost complete but with a trace of turbidity persisting.

It will be seen that 5 strains were completely agglutinated at 1-200 or over with "Clayton" serum; of these 1 was also complete at 1-100 with "Smith" serum, while the other 4 were negative with all other sera. Six strains were complete with Clayton serum at 1-100, and of these 4 were also complete at the same dilution with Smith serum, the other 2 with no other serum than Clayton.

One strain was complete at 1-200 with Boscombe and at 1-100 with Smith, while one was complete at 1-100 with Boscombe, and at 1-200 with Smith.

Three strains were complete at 1-200 and 12 at 1-100 with Smith.

Nine strains were not completely agglutinated with any serum, but showed some clumping at 1-100, 7 with Clayton serum, and 1 each with Boscombe and Chandler.

If these 9 are excluded as showing insufficient agglutination to justify their position as meningococci, there are 21 strains which could not readily be thrown out of the meningococcus category. Of these, 13-14 and 16-17 are pairs from the same throat at different examinations. There are, after deducting these, 19 individuals in whose nasopharynx organisms were found identical with or closely related to the

TABLE IV.

Titre of each serum with its own strain = complete agglutination at 1-600.

Strain	Boscombe			Clayton			Chandler			Smith		
	Highest dilution with complete agglutination	Highest dilution with agglutination = +	Agglutination at 1-100	Highest dilution with complete agglutination	Highest dilution with agglutination = +	Agglutination at 1-100	Highest dilution with complete agglutination	Highest dilution with agglutination = +	Agglutination at 1-100	Highest dilution with complete agglutination	Highest dilution with agglutination = +	Agglutination at 1-100
T.N. 1	-	-	trace	-	-	+	-	-	o	200	400	c
T.N. 2	200	400	c	-	100	++	-	-	o	100	200	c
T.N. 3	-	100	++	100	200	c	-	-	-	100	200	c
T.N. 4	-	-	o	-	-	+	-	-	o	-	-	o
T.N. 5	-	-	trace	100	200	c	-	-	o	-	-	+
T.N. 6	-	-	o	-	-	+	-	-	o	100	200	c
T.N. 7	-	-	+	100	200	c	-	-	o	100	200	c
T.N. 8	-	-	o	-	200	+++	-	-	o	-	-	+
T.N. 9	-	-	trace	-	100	++	-	100	++	200	300	c
T.N. 10	-	100	+++	-	-	o	-	-	o	-	-	+
T.N. 11	-	-	o	-	100	++	-	-	trace	100	200	c
T.N. 12	-	-	o	200	400	c	-	-	o	-	-	o
T.N. 13	100	200	c	-	100	++	-	-	o	200	400	c
T.N. 14	-	-	+	100	200	c	-	-	o	100	200	c
T.N. 15	-	-	o	200	400	c	-	-	o	100	200	c
T.N. 16	-	-	o	200	400	c	-	-	o	-	-	+
T.N. 17	-	-	o	400	800	c	-	-	o	-	-	+
T.N. 18	-	-	o	-	100	++	-	-	o	-	-	+
T.N. 19	-	-	o	-	-	+	-	-	o	-	-	+
T.N. 20	-	-	trace	200	400	c	-	-	o	-	-	trace
T.N. 21	-	-	o	-	-	+	-	-	+	100	200	c
T.N. 22	-	-	trace	-	100	+++	-	-	+	100	200	c
T.N. 23	-	-	trace	100	200	c	-	-	o	-	-	+
T.N. 24	-	-	o	-	100	+++	-	-	o	-	-	trace
T.N. 25	-	-	o	-	100	++	-	-	o	-	-	trace
T.N. 26	-	-	o	-	-	trace	-	100	+++	-	-	+
T.N. 27	-	-	trace	-	100	++	-	-	o	-	-	o
T.N. 28	-	-	o	-	100	++	-	-	o	100	200	c
T.N. 29	-	-	o	100	-	c	-	-	o	100	200	c
T.N. 30	-	-	trace	-	100	+++	-	-	o	100	200	c

meningococcus as shown by microscopical, cultural and serological tests; this is equivalent to 13.7 % of the throats examined at Lambeth.

Of the males examined (32), 15.6 % were positive, while 13.2 % of the females (106) were positive. Of those of 50 years and over 18.2 % were positive, while of those between 14 and 50 years 11.3 % were positive.

Only 1 "positive" was found among children under 14, of whom 19 were examined (5.3 %).

*Non-contact school children.*

Fifty-six children of ages from 5 to 13 attending a rural school were examined early in May. One plate only showed "suspicious" colonies, giving a strain whose morphological, cultural and fermentative characters were meningococcus-like. It gave a "trace" of agglutination with serum "Smith" at 1-100, but with no other serum.

*Agglutinin-Absorption Experiments.*

Further evidence of serological relationship of the above organisms to meningococci was sought by estimating their capacity of absorbing the specific agglutinins.

The growth was scraped from 48-hour glucose ascitic agar plates sown with the strains to be examined, weighed moist, mixed with Clayton serum diluted 1-50, so that each c.c. of the mixtures contained 40 mg. of growth. The mixtures were incubated 18 hours at 55° C., centrifuged, and the clear (or in some cases opalescent) fluid used as an agglutinating serum of 1-50 dilution. A control specimen of the diluted serum was similarly heated and centrifuged.

The results are recorded in Table V (p. 483).

The table shows that the non-contact throat strains T.N. 17 and T.N. 12, which are well agglutinated by Clayton serum, also absorb the agglutinin for the strain with which Clayton serum was prepared as well as the agglutinin for themselves, and this to almost the same extent as the homologous strain does with the same serum. Another strain, T.N. 4, which is agglutinated, as shown above, only to the smallest degree by Clayton serum, absorbs a just perceptible amount of agglutinin, while a non-agglutinating meningococcus strain, T. 1 (Boscombe), absorbed practically none.

Four other similar experiments were made with similar results which may be summarised thus: five known meningococcus strains and nine non-contact strains were treated with Clayton and with either Boscombe or Smith sera, *i.e.*, sera of another type; Clayton and Boscombe strains (which do not agglutinate at all with each other's sera) did not absorb agglutinin from each other's sera: agglutinability and absorptive capacity for agglutinin ran parallel: with the relatively weakly agglutinated strains increase in the quantity of the bacterial growth used for



TABLE V.

*Absorption of Agglutinin from Serum Clayton.*

Test Emulsions	Treatment of Serum	Agglutinations				
		1-100	1-200	1-400	1-600	1-800
T. 13 c (Clayton)	Control showing titre without exhaustion	c	c	c	c	+++
T.N. 17		c	c	c	+++	+
T.N. 12		c	c	++	+	o
T.N. 4		+	o	o	o	o
T. 13 c (Clayton)	Serum Clayton exhausted with T. 13 c (Clayton)	trace	o	o	o	o
T.N. 17		trace	o	o	o	o
T.N. 12		trace	o	o	o	o
T.N. 4		o	o	o	o	o
T. 13 c (Clayton)	Serum Clayton exhausted with T.N. 17.	+	o	o	o	o
T.N. 17		trace	o	o	o	o
T.N. 12		o	o	o	o	o
T.N. 4		o	o	o	o	o
T. 13 c (Clayton)	Serum Clayton exhausted with T.N. 12	+++	++	o	o	o
T.N. 17		++	o	o	o	o
T.N. 12		o	o	o	o	o
T.N. 4		o	o	o	o	o
T. 13 c (Clayton)	Serum Clayton exhausted with T.N. 4	c	c	+++	++	++
T.N. 17		c	c	++	+	o
T.N. 12		c	+++	+	+	trace
T.N. 4		o	o	o	o	o
T. 13 c (Clayton)	Serum Clayton exhausted with T. 1 (Boscombe)	c	c	c	c	++
T.N. 17		c	c	c	++	+
T.N. 12		c	c	+	+	o
T.N. 4		+	o	o	o	o

absorption increased the amount of agglutinin removed. There was no adequate evidence of a "group agglutinin" in the sense of one which could be removed by absorption with a related strain leaving the more "specific" agglutinin intact.

*Complement-Fixation Experiments with Strains from Cases of Cerebro-spinal Fever and from Non-contacts.*

Two sets of these were done: in the first, with an *extract* from meningococcal growths as antigen, the specific complement absorption was slight in amount and not in agreement in every case with the agglutination reactions. For example, T. 1 absorbed a little complement in combination with the non-agglutinating Clayton serum and no more with its homologous serum. T.N. 17, however, was consistent, as it

absorbed 5 doses of complement in combination with Clayton serum and less than one with Boscombe serum.

In the second experiment the antigens used were *heated emulsions* [65° C.], and the results were parallel as regards specificity with the agglutination tests; .05 c.c. of the agglutinating sera with .2 c.c. of bacterial emulsion took up 4 to 5 doses of complement, while the non-agglutinating sera in similar amounts took up 1 to 2 doses only.

#### SUMMARY.

(1) The maximum period during which meningococci may be isolated from the naso-pharynx of convalescents exceeds three months.

(2) Meningococci were isolated from the naso-pharynx and proved identical with those isolated from the spinal fluid of the same patient in seven cases.

(3) In two of these cases the type of meningococcus found in the naso-pharynx at first resembled exactly that found in the spinal canal of the same patient, and later was persistently replaced by a meningococcus differing markedly in serological reactions from the spinal strain.

(4) Micro-organisms indistinguishable from meningococci by microscopical and cultural methods (including fermentation tests) were found in the naso-pharynx in 22 % of 138 individuals, non-contacts, from an urban population (Lambeth out-patients).

(5) With 63 % of these organisms the serological tests confirmed their identity with or close relationship to meningococci; they agglutinated specifically with anti-meningococcus serum and exhibited a tendency to fall into the same serological groups as the spinal strains.

(6) Their agglutinating properties were not, in general, so strongly marked with the sera used as those of the known pathogenic strains but they showed definite absorption of the specific agglutinin. They appeared to differ from the majority of the spinal strains not in the quality but in the quantitative intensity of their specific affinities: some spinal strains, however, resembled them in this.

(7) Thus, in 13.7 % of the 138 non-contacts micro-organisms were found in the naso-pharynx indistinguishable by any test from strains of meningococci known to have caused meningitis: these are regarded by the writer as meningococci and the individuals harbouring them as meningococcus-carriers.

## THE MECHANISM OF THE AGGLUTINATION REACTION.

BY HENRY PRIESTLEY, B.Sc., M.D., CH.M.

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SINCE Gruber and Nicolle expressed the opinion that the agglutination of bacteria by specific immune sera was due to the surfaces of the bacteria becoming viscous under the influence of the specific sera, many theories have been advanced aiming at a more complete explanation of the reaction. Bordet (1899) considered that the agglutinins by uniting with the agglutinable substances lead to changes in molecular attraction between the elements affected, either as among themselves, or between them and the surrounding fluid. He distinguished the two phases of the reaction, (1) the interaction between the agglutinin and the bacteria, and (2) the agglomeration of the bacteria.

Kraus (1897) observed that the addition of specific agglutinating sera to bacterium-free filtrates of broth cultures of cholera vibrios, typhoid and plague bacilli resulted in the formation of a precipitate, and according to Nicolle (1898) watery extracts of agar cultures of *B. typhosus*, *B. coli* and *V. cholerae* gave a precipitate with the appropriate antiserum. He showed further that even non-specific bacteria, added to a mixture of antiserum and extract of homologous bacteria, were completely agglutinated. In these experiments, however, he did not use high dilutions of either serum or extract.

Paltauf (1897) based his theory of agglutination on these observations. According to him the agglutination of bacteria by a specific serum is due to the formation of a coagulum outside the bacteria. This coagulum, which is the result of the interaction of agglutinin and agglutinable substance diffused from the bacterial cells, draws the bacteria together mechanically.

Löwit (1903) and later Arkwright (1914) claim to have demonstrated a coagulum around agglutinated bacteria, but a number of other workers have been unable to confirm these observations.

Recently Arkwright (1914) has brought forward further evidence in favour of Paltanuf's hypothesis. He found that if a mixture were made of extract of typhoid bacilli in distilled water, typhoid immune serum and non-specific bacteria, such as *B. coli* or *B. acidi lactici*, or even particles of animal charcoal or kieselguhr, the bacteria or inorganic particles were agglutinated with very considerable dilutions of the antiserum and extract. Thus in a mixture of extract of typhoid bacilli, typhoid immune serum and *B. coli* he obtained definite macroscopic agglutination of the *B. coli* with a dilution of 1 : 27,000 serum and 1 : 96 extract. Control experiments showed that in these dilutions no visible precipitate was formed from the interaction of the serum and extract alone, and the *B. coli* were not agglutinated by the serum alone. If a mixture of extract and antiserum in certain concentrations were made, a visible precipitate was formed; the addition of normal serum in certain proportions to such a mixture increased the bulk of the precipitate.

Previously Dean (1912) had shown that the addition of a small quantity of a solution of euglobulin—that part of the serum proteins which is precipitated by slightly acidifying a dilution of serum in distilled water—to a mixture of horse serum, increased the quantity of the precipitate, and there was visible precipitation with higher dilutions of antiserum in the presence of the euglobulin than in its absence. Further, specific antiserum caused agglutination of bacteria in higher dilutions when a small quantity of euglobulin solution, or of normal serum, was added.

Moreschi (1908) found that the addition of homologous precipitating antiserum to a mixture of bacteria and specific agglutinating serum increased the agglutination titre of the specific serum to a greater degree than the addition of normal serum. Thus in one experiment using human typhoid immune serum he found that a dilution of 1 in 40 gave agglutination with *B. typhosus* with the addition of 0.05 of normal rabbit serum, and 1 : 1280 gave agglutination with the addition of 0.05 of antihuman rabbit serum.

These observations suggest strongly that the agglutination of bacteria by a specific immune serum is due to the formation of a precipitate outside the bacteria, which in some way causes the bacteria to clump together and become agglomerated.

The experiments of Kraus, of Nicolle and of Arkwright show that a precipitate is formed as the result of the interaction between bacterial extract and specific immune serum and that this precipitate will cause the agglomeration of non-specific bacteria.



In Moreschi's experiments a precipitate is formed in the interacting mixture as a result of the interaction between the human serum and the antihuman serum, and it seems justifiable to assume that it is this precipitate which increases the agglutinating titre of the human typhoid immune serum.

The result of Dean's experiments may be correlated with those of Arkwright, and again it seems justifiable to assume that the increase in titre of the agglutinating serum is due to the increased formation of precipitate.

The conception that the agglutination of bacteria is brought about by the formation of a precipitate is strengthened by the observations of Scheller (1910). He found that if a mixture of bacteria and specific immune serum were shaken shortly after the commencement of the reaction, the agglutination of the bacteria might be completely inhibited.

He considered that the disagglutination was due to the rendering homogeneous of the precipitate which he supposed to be the cause of the agglutination.

It has for a long time been recognised that the agglutination reaction and the precipitin reaction present many analogies.

The agglutinins as well as the precipitins show the phenomenon of inactivation and the production of an inhibitory property on heating at certain temperatures.

In both the precipitin and agglutination reactions, as Bordet pointed out, the presence of sodium chloride, or at any rate of an electrolyte, is necessary for the agglutination.

Both precipitins and agglutinins are intimately connected with the globulin fraction of the serum, and are carried down completely when the globulins are precipitated by magnesium sulphate or ammonium sulphate. Further the denaturation of the globulin is accompanied by complete destruction both of agglutinin and precipitin.

In the precipitin reaction Welsh and Chapman (1906) have shown that the greater part, if not the whole of the precipitate, is derived from the antiserum and not from the antigen as previously believed, and that antigen is not removed in appreciable amounts from the mixture of antigen and antiserum by the precipitate. They attempted to exhaust the antigen in solution by the repeated addition of antiserum, and found that the sixth successive addition gave as much precipitate as the first, although the amount of antigen could not, on account of the dilution, be more than one-tenth of that originally present, assuming that none of it was removed in the precipitate.

I have found that bacteria and specific agglutinating sera gave analogous results. A thick emulsion of *B. typhosus* in normal saline solution was mixed with typhoid goat antiserum, so that the dilution of antiserum was 1:10. After twenty-four hours the agglutinated bacteria were centrifuged off completely and the supernatant fluid removed. A further quantity of antiserum was added to the superfluid and after twenty-four hours there was a large precipitate which was separated. This process was repeated four times and there was still a marked precipitate after the fourth addition of antiserum.

This experiment was repeated a number of times with immune sera from different animals. Microscopical examination of the precipitate showed it to consist of aggregates of amorphous material. From this it is evident that the antigen was not completely removed with the agglutinated bacteria, but that part of the antigen had diffused into the surrounding fluid and that the precipitate was largely, if not entirely, derived from the serum added.

There are, however, apparent differences between the two reactions. There is, for example, not complete agreement between the phenomenon of agglutination of bacteria, and that of the production of a precipitate with filtered broth cultures of the same bacteria, by specific sera. Numerous examples have been given by different workers where the formation of agglutinins in a serum has not run parallel with the formation of bacterio-precipitins for the broth cultures of the bacteria.

In my opinion this discrepancy may be explained in the following manner:

While it is undoubted that a precipitate is formed outside the bacterial bodies in an agglutination reaction, it is probable that as bacteria absorb protein from serum, precipitation takes place also within the cells, or at any rate in extremely close relationship to the cell walls.

Joos (1903) and others have shown that agglutinin and agglutininogen are not uniform substances but consist of a number of different components. It seems, therefore, likely that the antigen for one or more components of the agglutinins is retained within the bacteria, because non-diffusible, and in consequence the interaction of antigen and antibody will, in part, take place within the bacterial cell or in the cell wall. Moreover it is not likely that antibodies to different parts of the cell protoplasm are formed at different rates so that serum taken from an animal which is being immunised may at one time give a sufficient precipitate with the antigen retained in the bacterial cell and not with

the diffusible antigen, while at another time it may give a marked precipitate with both.

That the formation of a precipitate within the bacterial cell can give rise to the agglutination of bacteria is shown by the experiments of Neisser and Friedemann (1904). These authors treated bacteria with lead salts, then washed them till the washings contained no trace of lead. The addition of sulphuretted hydrogen water caused immediate strong agglutination and the bacteria were stained black.

Another difficulty which has been advanced in connection with the conception of the identity of the agglutination and precipitin reactions, is the observation that agglutinating sera may produce agglutination in extremely high dilutions while precipitin sera only produce visible precipitates if a relatively large amount of the antiserum be employed.

In a mixture of antigen and a relatively small amount of precipitin serum, the individual molecules of serum protein are widely separated so that the formation of aggregates of sufficient size to be visible may be impossible, besides, the amount of serum protein present may be too small to be visible on separation. The close analogy which exists between the precipitin and agglutination reactions suggests that the agglutination is due to the precipitation of a certain fraction of the serum proteins in intimate relationship to the bacteria. The relatively great size of the bacterium serum protein complex, however, affords ample opportunity for the particles of serum protein to approach one another, and with the bacteria to form aggregates of sufficient size to produce a visible precipitate.

#### RELATION OF AGGLUTINATION TO THE GLOBULIN OF THE SERUM.

Franceschalli (1909) has shown that in a particular series of precipitin reactions 42·8 per cent. of the globulin of the antiserum was removed in the precipitate.

Dean (1912) has shown that the addition of euglobulin solution to a mixture of bacteria and antiserum increases the titre of the antiserum considerably; in an analogous way the amount of precipitate in a precipitin reaction is increased by the addition of euglobulin solution. He considers that the interaction of the antigen and antibody causes an aggregation of the molecules of a non-specific substance, which is possibly serum globulin, and that this aggregation is an essential part of the process of agglutination.

It seems probable that the greater part at least of the precipitate in a precipitin reaction consists of serum globulin, derived from the antiserum, which is altered in some way and is not necessarily specific.

From analogy, the precipitate which causes the agglutination reaction may also consist of altered globulin with or without some other altered serum proteins, hence agglutinated bacteria should show similar properties to altered or "denaturated" proteins. That this is the case is shown in part by Tullock's (1914) experiments. He agglutinated *B. paratyphosus* B with specific antiserum, washed them twice with distilled water and resuspended in distilled water. A considerably smaller amount of a divalent salt such as calcium chloride or barium chloride than of a monovalent salt such as sodium chloride was needed to produce reagglutination. Chick and Martin (1912) obtained similar results with denaturated serum proteins and denaturated egg albumin in faintly alkaline dispersions.

In the case of acid dispersions of washed agglutinated bacteria Tullock found that the agglutinating value of a salt depended on the valency of the anion as did Chick and Martin in the agglutination of acid dispersions of denaturated proteins; thus sodium sulphate was more active than sodium chloride or barium chloride.

It seems probable, then, that the agglutination of bacteria by specific antiserum is, in the main, due to the formation of altered serum globulin in and around the bacteria and the subsequent flocculation by electrolytes of this altered globulin together with the entangled bacteria.

#### EFFECTS OF ACIDS AND ALKALIS ON AGGLUTINATION.

The effects of low concentrations of acids and alkalis on denaturated proteins have been studied in some detail by several workers, notably Chick and Martin.

##### (a) *Effects of dilute acids and alkalis on the agglutination of bacteria by specific sera.*

If acids and alkalis be added, in sufficient amount, to a mixture of bacteria and specific agglutinating serum, the agglutination of the bacteria is inhibited. Alkalis have a somewhat more marked effect, in this direction, than have acids, as will be observed in Table I, where 0.10 cc. of N/100 potassium hydrate produced some inhibition, while the same amount of N/100 sulphuric acid was without effect.



TABLE I.

Goat *v.* typhoid serum diluted 1:10 in normal saline solution 0.1 cc.

Emulsion of 24 hours agar culture of *B. typhosus* in normal saline solution 0.5 cc.  
4 per cent. sodium chloride solution 0.1 cc.

N/100  $\text{H}_2\text{SO}_4$  or N/100 KOH in each tube in amount indicated.

Contents of all tubes made up to 1 cc. with distilled water.

2 hours at 37° C. 18 hours at room temperature.

	0	.02	.04	.06	.08	.10	.12	.14	.16	.18	.20	.22
N/100 $\text{H}_2\text{SO}_4$	+++	+++	+++	+++	+++	+++	+++	++	+	-	-	-
N/100 KOH	+++	+++	+++	+++	+++	++	++	+	+	-	-	-

(b) *Effect of the nature of the acid.*

TABLE II.

Rabbit *v.* typhoid serum 1:10 in saline, 0.1 cc. in each tube.

Emulsion of 24 hours agar culture of *B. typhosus*, 0.5 cc.

N/10  $\text{H}_2\text{SO}_4$ , N/10  $\text{H}\bar{\text{A}}$  and N/10  $\text{H}_2\bar{\text{T}}$  or equivalent added in amounts indicated

Contents of each tube made to 1 cc. with distilled water.

2 hours at 37° C. 24 hours at room temperature.

N/10 $\text{H}_2\text{SO}_4$	...	0	.010	.012	.014	.016	.018	.020	.022	.024
		+++	+++	+++	+++	+++	+++	+++	+++	+++
N/10 $\text{H}\bar{\text{A}}$	...		.016	.018	.020	.022	.024	.026	.028	.030
			+++	+++	+++	+++	+++	+++	+++	+++
N/10 $\text{H}_2\bar{\text{T}}$	...		.16	.18	.20	.22	.24	.26	.28	.30
			+++	+++	+++	+++	+++	+++	+++	+++
N/10 $\text{H}_2\text{SO}_4$	...	.026	.028	.030	.032	.034	.036	.038	.040	
		++	+	+	-	-	-	-	-	
N/10 $\text{H}\bar{\text{A}}$	...	.032	.036	.038	.04	.06	.08	.10		
		+++	+++	+++	+++	++	+	-		
N/10 $\text{H}_2\bar{\text{T}}$	...	.32	.34	.36	.38	.40				
		+++	+++	+++	+++	+++				

The nature of the acid used has a marked influence. Thus in Table II it is seen that sulphuric acid inhibited in a lower concentration than did acetic acid, and tartaric acid had a feeble inhibitory effect. Although tartaric acid, in the dilutions used, did not inhibit the agglutination as observed at the end of twenty-four hours there was a considerable slowing of the reaction, and at the end of four hours there was no agglutination in the tubes containing more than 0.3 cc. N/10 tartaric acid.

This result is analogous to the results obtained by Chick and Martin in the agglutination of denaturated serum proteins.

It is necessary to point out here that suspensions of denaturated proteins are agglutinated if acids be added in certain concentrations. If more acid be added the agglutinated proteins are again dispersed.

Chick and Martin found that the weakest acid—butyric acid in a particular experiment—gave a wider range within which the denaturated protein was agglutinated, than the stronger acids—acetic and hydrochloric. When the acids reached a certain concentration the protein was again dispersed, and a much greater equivalent amount of the weaker acid was needed than of the stronger to cause dispersion. These results, they maintain, are due to the differences in the H-ion concentration of equivalent dilutions of the acids.

(c) *Effects of relative proportions of acids or alkali and serum.*

Table III shows that in order to inhibit agglutination, the acid or alkali and the serum must be present in certain relative proportions. The greater the quantity of serum present, the greater is the amount of acid or alkali required to inhibit agglutination.

TABLE III.

Typhoid v. goat serum 0.1 cc., 0.01 cc., 0.001 cc. respectively in each tube in the three series.

0.5 cc. emulsion of *B. typhosus* in normal saline.

N/100  $\text{H}_2\text{SO}_4$  in amounts indicated.

Contents of each tube made up to 1 cc. with distilled water. Results after 24 hours at room temperature.

N/100 $\text{H}_2\text{SO}_4$ ...	0	0.08	0.10	0.12	0.14	0.16	0.18	0.20	0.22	0.24
0.1 cc. serum ...	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.01 cc. „ ...	+++	+++	+++	+++	+++	+++	+++	++	++	+
0.001 cc. „ ...	+++	+++	+++	++	++	++	+	+	+	+
0 ...	—	—	—	—	—	—	—	—	—	—

Thus in the experiment detailed in Table III it was found that using 0.1 cc. of agglutinating serum, 0.24 cc. of N/100 sulphuric acid was insufficient to cause any inhibition, while with 0.001 cc. of serum there was almost complete inhibition with 0.18 cc. N/100 sulphuric acid. This may in part be due to adsorption of acid by serum proteins other than those concerned in the agglutination of the bacteria, but another explanation will be given later.

*(d) Effect of absolute concentration of acid or alkali.*

If serum be mixed with acids or alkalis and a series of dilutions of the mixture made and added to a suspension of homologous bacteria, a dilution will be reached where there is complete agglutination in spite of the presence of acids or alkali, provided that not too much acid or alkali has been added (see Table IV). From this it is evident that a certain absolute concentration of acid is necessary to produce inhibition.

TABLE IV.

Rabbit *v.* typhoid serum 1:10 in saline solution mixed with an equal volume of N/10  $\text{H}_2\text{SO}_4$  and following dilutions made with normal saline solution, the dilution being reckoned in terms of whole serum present in final mixture of serum, acid and bacterial emulsion.

Emulsion of *B. typhosus* in normal saline solution 0.5 cc. in each tube.

Contents of all tubes made to 1 cc. with saline. 3 hours incubator.

Serum and acid	1:100	1:200	1:400	1:600	1:800	1:1600	1:3200	1:6400
	-	-	-	-	+++	+++	++	-

This point is brought out also in another experiment in which different quantities of acid were added to a mixture of bacteria and a series of dilutions of specific immune serum (see Table V). It is seen that 0.4 cc. of N/100 sulphuric acid almost completely inhibited agglutination by 1/100 serum, while 0.04 cc. had no inhibitory action whatever.

TABLE V.

Rabbit *v.* typhoid serum in dilutions indicated.

Emulsion of *B. typhosus* in normal saline solution 0.5 cc. in each tube.

Amounts of N/100  $\text{H}_2\text{SO}_4$  as indicated.

Contents of each tube made up to 1 cc. with distilled water.

2 hours at 37° C. 24 hours room temperature.

*Serum.*

		1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400	0
N/100 $\text{H}_2\text{SO}_4$	0.04 cc.	+++	+++	+++	+++	+++	++	+	-
	0.2 cc.	+++	++	+	+	+	+	+	-
	0.4 cc.	+	+	+	+	+	-	-	-
No acid	...	+++	+++	+++	+++	+++	++	+	-

*(c) Time factor in acid or alkali inhibition.*

The interval of time elapsing between the addition of the acid or alkali and the mixing of the bacteria and serum is of importance. If the acid or alkali be added at the time the mixture of serum and bacteria is made, much less is required to inhibit the agglutination than if either be added some time later, and within certain limits the longer the time interval the greater is the amount of acid or alkali required. It will be seen in Table VI that if the amount of acid or alkali added be kept constant, inhibition is observed in the presence of greater concentrations of serum, if the addition be made at the time of mixing than if it be made later.

TABLE VI.

Rabbit *r.* typhoid serum in dilutions indicated.  
 Emulsion of 24 hours agar culture of *B. typhosus* in normal saline solution, 0.5 cc. in each tube.  
 0.1 cc. N/10 KOH added to each tube at stated intervals.  
 Contents of each tube made up to 1 cc. with normal saline solution.  
 3 hours at 37° C. after last addition. 24 hours at room temperature.

<i>Serum.</i>									
Alkali added	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
After 0 minutes	+++	+++	—	—	—	—	—	—	—
„ 30	+++	+++	+++	+++	+	—	—	—	—
„ 60	+++	+++	+++	+++	+++	+	—	—	—
„ 120	+++	+++	+++	+++	+++	+++	+++	+	—
Control	+++	+++	+++	+++	+++	+++	+++	+++	++

An explanation is probably to be found in the different states of aggregation of the agglutinated bacteria at different times after the commencement of the agglutination reaction. The precipitation of colloids from solution or suspension by electrolytes is apparently due to the adsorption by the particles of that ion of the electrolyte which bears an electric charge opposite in sign to their own. Particles bearing charges of the same sign repel one another. The adsorption of ions of opposite sign neutralises the charge carried by the particles and they are no longer mutually repelled, but come together and form aggregates which fall to the bottom of the containing vessel, the rate of fall depending on the size of the aggregates. If the charge given to the particles by the adsorbed ions be greater in amount than is necessary to neutralise the charge already borne by them, they will take on the opposite charge to that which they originally bore and will again repel one another and hence be dispersed.



The aggregates of agglutinated bacteria can readily be observed to become larger and more coherent with the lapse of time, the rate at which this occurs depending, among other things, on the amount of agglutinating serum which is present.

Chick and Martin (1912) point out that the dispersion of particles of denaturated proteins by small amounts of acid or alkali, is due to the electric charge given to the particles. The particles of a loose aggregate can more readily take on this charge and be dispersed by it than the particles of a coherent mass, for in the latter case the aggregate is probably charged as a whole and any disruptive action is limited to the superficial particles and even here operates at great mechanical disadvantage.

The effect of acid or alkali in preventing agglutination may be explained in a similar way; the more coherent the aggregate of bacteria is, the smaller is the surface on which the charge can act. It follows, therefore, that the greater the degree of cohesion of the agglutinated bacteria, the greater is the charge necessary to disagglutinate them and hence the greater the amount of acid or alkali required. With a small amount of agglutinating serum the rate at which cohesion takes place is lower than with a large amount of agglutinating serum.

TABLE VII.

Typhoid bacilli from agar culture were agglutinated with (A) 1-10, (B) 1-100, (C) 1-1000 typhoid goat serum and after 4 hours were centrifuged and washed twice with distilled water then re-emulsified in distilled water. In each tube was placed 0.5 cc. of the respective emulsions, the indicated amount of N/100 H<sub>2</sub>SO<sub>4</sub> or equivalent and distilled water to make the contents of each tube up to 1 cc.

N/100 H <sub>2</sub> SO <sub>4</sub>	·002	·004	·006	·008	·010	·012	·014	·016	·018
A 1- 10 ...	++	++	++	++	+++	+++	+++	+++	+++
B 1- 100 ...	-	-	-	-	-	+	++	+++	+++
C 1-1000 ...	-	-	-	-	-	-	-	-	-
N/100 H <sub>2</sub> SO <sub>4</sub>	·020	·04	·06	·08	·10	·12	·14	·16	0
A 1- 10 ...	+	+	+	+	+	-	-	-	-
B 1- 100 ...	+++	++	+	+	+	-	-	-	-
C 1-1000 ...	+	+++	+++	+++	++	+	+	-	-

*Effects of acids on washed agglutinated bacteria.*

If bacteria be agglutinated by specific sera, then washed free from serum and salts and emulsified in distilled water, they remain dispersed for a considerable length of time without re-agglutination taking place. The addition of low concentrations of acids to such dispersions may, however, cause re-agglutination.

The amount of acid required to re-agglutinate the bacteria depends upon the amount of agglutinating serum originally used to "sensitise" the bacteria. Numerous observers have shown that unsensitised bacteria may be agglutinated by certain concentrations of acids. The strain of *B. typhosus* used in these experiments showed partial agglutination between the limits of 0.06 cc. and 0.18 cc. of N/100 sulphuric acid when unsensitised. It is seen in the experiment detailed in Table VII that when the bacteria were sensitised with 1 : 10 serum 0.01 cc. to 0.018 cc. of N/100 sulphuric acid caused re-agglutination of the washed bacteria, while when they were sensitised with 1 : 1000 serum 0.04 cc. to 0.08 cc. of N/100 acid were required to cause re-agglutination. It is evident, then, that with the smaller amount of serum more acid is required than with the large.

Analogous results were obtained by Porges (1906), using sodium chloride in place of acid. Thus serum diluted 1 : 10 gave agglutination in the presence of 0.0002N sodium chloride, while serum 1 : 2000 required 0.02N sodium chloride for agglutination.

Tullock (1914) found that emulsions of *B. suipestifer*, with 1 : 500 antiparatyphosus B serum required the presence of N/80 sodium chloride to be agglutinated while *B. paratyphosus* B was agglutinated with the same dilution of serum in the presence of N/400 sodium chloride. This is evidently due to the presence of a smaller amount of group agglutinin which agglutinates *B. suipestifer*, than of the specific agglutinin.

TABLE VIII.

Calf serum diluted 1 : 10, 1 : 50 and 1 : 100 respectively in distilled water boiled for a few seconds. 0.5 cc. serum dilution in each tube. Amount of N/100 H<sub>2</sub>SO<sub>4</sub> or equivalent as indicated. Contents of all tubes made up to 1 cc. with distilled water.

N/100 H <sub>2</sub> SO <sub>4</sub>	...	0	·02	·04	·06	·08	·10	·12	·14	·16
Serum 1 : 10	...	—	—	—	—	c	+++	+++	+++	+++
N/100 H <sub>2</sub> SO <sub>4</sub>	...	0	·002	·004	·006	·008	·010	·012	·014	·016
Serum 1 : 50	...	—	—	—	—	—	—	—	—	—
N/100 H <sub>2</sub> SO <sub>4</sub>	...	0	·002	·004	·006	·008	·010	·012	·014	·016
Serum 1 : 100	...	—	—	—	—	—	sc	c	+++	c
N/100 H <sub>2</sub> SO <sub>4</sub>	...	·18	·20	·22	·24	·26	·28	·30	·32	·34
Serum 1 : 10	...	+	sc	sc	—	—	—	—	—	—
N/100 H <sub>2</sub> SO <sub>4</sub>	...	·018	·02	·04	·06	·08	·10	·12	·14	·16
Serum 1 : 50	...	sc	c	+++	—	—	—	—	—	—
N/100 H <sub>2</sub> SO <sub>4</sub>	...	·018	·02	·04	·06	·08	·10	·12	·14	·16
Serum 1 : 100	...	c	sc	—	—	—	—	—	—	—

+++ = Large precipitate, fluid clear.

++ = Slight precipitate, fluid cloudy.

c = Cloudy. No precipitate.

sc = Slightly cloudy.

— = Clear. No precipitate.

As an explanation of these observations the following is suggested:

The agglutination of bacteria is dependent on two opposing tendencies: (1) the tendency of the bacteria to remain dispersed; (2) the tendency of the agglutinin to cause aggregation of the bacteria.

The aggregation of bacteria "sensitised" by agglutinating serum is probably due to the neutralisation of the electric charge carried by the bacterium-serum protein complexes, just as the aggregation of particles of denaturated protein is occasioned by neutralisation of the charges originally carried by them.

When a high dilution of agglutinating serum is used to agglutinate bacteria a smaller amount of serum protein is fixed in relationship to the bacteria than when a lower dilution is used. If "sensitisation" of the bacteria be accompanied by a partial electric discharge following the formation of the bacterium-serum protein complex, it follows that a larger amount of acid will be necessary to produce aggregation when there is little protein fixed than when there is much. For, in the former case, the force exerted to prevent aggregation will have been neutralised by the serum protein fixed to a less degree than in the latter.

The amount of acid required to agglutinate denaturated serum protein depends upon the amount of protein present as shown in Table VIII. It is seen that serum diluted 1:10 with distilled water and boiled required the addition of 0.1 to 0.16 cc. of N/100 sulphuric acid to cause agglutination while serum diluted 1:100 required only 0.014 cc. of N/100 acid.

TABLE IX.

Precipitate from the interaction of calf serum and goat *v.* calf serum was washed twice with distilled water and re-emulsified in distilled water; 0.5 cc. emulsion in each tube and amounts of N/1000 H<sub>2</sub>SO<sub>4</sub> as indicated. Contents of all tubes made to 1 cc. with distilled water.

N/1000 H <sub>2</sub> SO <sub>4</sub>	...	0	·02	·04	·06	·08	·10	·12	·14	·16
		-	+++	+++	++	+	-	-	-	-

The precipitate in a precipitin reaction if washed free from serum and salts and emulsified in distilled water, acts in a similar fashion to agglutinated bacteria in the presence of acids, being precipitated by small concentrations of acids and redispersed by greater concentrations (see Table IX).

Heated serum euglobulin acts also in a similar manner. Euglobulin was prepared from calf serum by diluting 1:10 in distilled water and saturating with carbon dioxide. After half an hour the

precipitate of euglobulin, which was formed, was separated, washed with and emulsified in distilled water forming a cloudy suspension. One half of the suspension was boiled for a few seconds after having been thoroughly shaken in order to disperse the particles completely. The heated euglobulin suspension has been kept for weeks without showing any sign of precipitation.

TABLE X.

In each tube 0.5 cc. of either heated or unheated euglobulin suspension in distilled water and N/100  $\text{H}_2\text{SO}_4$  or equivalent in amounts indicated.

Contents of all tubes made up to 1 cc. with distilled water.

N/100 $\text{H}_2\text{SO}_4$ ...	·002	·004	·006	·008	·010	·012	·014
Unheated euglobulin	T	T	T	T	+++	+++	+++
Heated euglobulin ...	T	T	T	+	++	+++	+++
N/100 $\text{H}_2\text{SO}_4$ ...	·016	·018	·020	·04	·06	·08	
Unheated euglobulin	+++	+++	c	c	c	c	
Heated euglobulin ...	+	+	T	T	T	T	

T = turbid.

c = clear.

+++ = large precipitate.

++ = large precipitate with marked turbidity.

+ = small precipitate.

It will be seen in Table X that the addition of more acid than was required to agglutinate the unheated euglobulin caused the suspension to clear completely. In the case of the heated euglobulin, on the other hand, there was a zone in which agglutination occurred, the tubes containing a smaller amount of acid than 0.008 cc. or a greater amount than 0.02 cc. being equally turbid. The resemblance in the appearance of the contents of the tubes in the heated euglobulin series to those of an analogous series using washed agglutinated bacteria was remarkably close.

TABLE XI.

In each tube was placed 0.5 cc. of the heated serum + bacteria (S + B) mixture, of the heated serum (S), or of the heated bacterial suspension (B).

N/100  $\text{H}_2\text{SO}_4$  was added in the amounts indicated and the contents of each tube made up to 1 cc. with distilled water.

N/100 $\text{H}_2\text{SO}_4$ ...	0	·10	·12	·14	·16	·18	·20	·22	·24	·26
S	...	-	-	cloudy	++	+++	+++	+++	+++	+++
S + B	...	-	-	-	++	+++	++	++	+	+
B	...	-	-	-	-	-	-	-	-	-

+++ = complete agglutination or large precipitate.

++ = large amount of agglutination or precipitate not so large.

+ = slight precipitate.

- = no agglutination or no precipitate



The precipitation of denaturated serum proteins by acids may occasion the aggregation of bacteria suspended in the mixture. An emulsion of *B. dysenteriae* was made in calf serum diluted 1:10 with distilled water and after standing for five minutes, the emulsion was boiled for a few seconds. The same serum diluted 1:10 in distilled water and an emulsion of *B. dysenteriae* in distilled water were also boiled for a few seconds. The *B. dysenteriae* was not agglutinated by the unheated calf serum in any dilution.

It will be seen in Table XI that there was only one tube in which the bacteria were completely agglutinated so that the formation of a large precipitate of denaturated protein does not necessarily result in the carrying down of the bacteria. This probably has to do with the rapidity of aggregation of the particles of denaturated protein. If the particles be aggregated rapidly, as they were in the tubes with the larger amounts of acid, the bacteria probably have less chance of becoming entangled in the precipitate, than if they be aggregated more slowly.

Attention may be drawn in this connection to the observations of H. R. Dean (1911) on the deviation of complement in precipitin reactions. He found that if a precipitate be formed rapidly, there was little, if any, deviation of complement while a very slowly forming precipitate deviated a considerable amount of complement.

A consideration of the experiments detailed in Tables VII–XI show that there is an undoubted analogy in the results obtained. Bacteria “sensitised” by specific agglutinating serum, precipitate from a precipitin reaction, and denaturated englobulin were all agglutinated by low concentrations of acids and dispersed by higher concentrations and all showed a range of optimum acidity for agglutination. The similarity of the results would seem to justify the assumption that the reactions are expressions of the same process and strengthens the conception that the precipitin reaction is due, in the main, to the precipitation of altered serum globulin and that agglutination of bacteria in an agglutination reaction follows as a sequence to the precipitation of altered serum globulin.

#### *Effects of heat on the properties of agglutinating serum.*

A considerable amount of work has been done on the effect of high temperatures on agglutinating serum.

It is found that if agglutinating sera be heated for half an hour at certain temperatures—varying according to the serum employed but

usually in the vicinity of 60°–70° C.—the serum may no longer produce agglutination in any dilution. If the serum be heated for half an hour, at a higher temperature—usually about 75° C.—the serum may become, not only inactive, but also inhibitory, that is the addition of a sufficient amount to a mixture of unheated serum and bacteria, will inhibit the agglutination. Heating at still higher temperatures will destroy this inhibitory effect, while the serum still remains inactive.

TABLE XII.

Goat *v.* typhoid serum diluted 1:10 with distilled water.

Parts of the dilution were heated at 75° C. in a water bath for 10 minutes, 20 minutes and 30 minutes respectively.

Dilutions of serum as indicated, the dilutions being given in terms of undiluted serum.

Emulsions of 24 hours agar culture of *B. typhosus* 0.5 cc. in each tube.

Contents of each tube made up to 1 cc. with saline.

4 hours at 37° C. Room temperature 18 hours.

		·01	·005	·0025	·00125	·000625	·000312	·000156	·000078
Heated	0 minutes	+++	+++	+++	+++	++	+	—	—
..	10 ..	+	++	++	+	—	—	—	—
..	20 ,	—	+	+	—	—	—	—	—
..	30 ..	—	—	—	—	—	—	—	—

If different lots of a dilution of serum be heated for different times, another phenomenon may be observed—the production of zones of inactivation. It is seen in Table XII that in this particular experiment heating for twenty minutes did not inactivate the serum completely, but that complete inactivation was observed in low and high dilutions, 1/100 and 1/800, but incomplete in a middle zone. In another experiment where 1:5 goat *v.* typhoid serum was heated at 77° C. for twenty minutes, there was complete agglutination with 0.0025 cc. of serum, but only a trace with 0.02 cc.

It is probable that in these cases, the inactivation is due to the presence of inhibitory substance in sufficient amounts in the high concentrations to produce inhibition.

Serum heated but not completely inactivated may, in certain concentrations, inhibit the agglutination by unheated serum (see Table XIII).

Heated immune sera vary considerably in their ability to produce inhibition. Some heated sera will inhibit the agglutination by an equal concentration of unheated serum, provided the dilution is not great, while other heated sera must be added in much greater amounts to cause inhibition. Thus, in the experiment detailed in Table XIII, 0.02 cc. of heated serum completely inhibited the agglutination by 0.00125 cc. of unheated serum, but not of 0.0025 cc.

TABLE XIII.

Rabbit *v.* typhoid serum 1:5 in distilled water heated at 75° C. in a water bath for half an hour.

Dilutions of serum in terms of original serum.

0.5 cc. emulsion of *B. typhosus* in each tube.

Contents made up to 1 cc. with normal saline solution.

3 hours at 37° C. 18 hours at room temperature.

	0.08	.01	.005	.0025	.00125	.000625	.000312	.000156	.000078	.000039
Unheated serum	.	+++	+++	+++	+++	+++	+++	++	+	-
Ditto + 0.02 cc. heated serum	.	+++	++	+	-	-	-	-	-	-
Ditto + 0.08 cc. heated serum	.	-	-	-	-	-	-	-	-	-
Heated serum	-	+	++	++	++	++	++	++	+	-

Eisenberg (1906) showed that high concentrations of heated sera inhibited agglutinations to a greater extent than low concentrations. Thus he found that heated serum 1/100 did not completely inhibit unheated serum 1/500, while heated serum 1/10 almost completely inhibited unheated serum 1/10.

There is a close resemblance between the effects of heated serum and the effects of acids and alkalis in preventing agglutination.

TABLE XIV.

Goat *v.* typhoid serum in dilution 1:5 in distilled water heated at 75° C. in water bath for half hour 0.4 cc. heated serum dilution added to each tube of series 1, 2 and 3 after intervals noted and 0.4 cc. distilled water to series 4.

Dilutions of unheated serum as indicated.

0.5 cc. emulsion of 24 hours agar culture of *B. typhosus* in normal saline solution in each tube.

	.05	.025	.0125	.00625	.00312	.00156	.00078	.00039	.00019
Heated serum added at once	+++	+++	+++	+++	+	-	-	-	-
After 10 minutes	+++	+++	+++	+++	+++	+++	+	-	-
„ 60 „	+++	+++	+++	+++	+++	+++	+++	+	-
No heated serum	+++	+++	+++	+++	+++	+++	+++	+++	++

Thus in both cases the zone phenomenon may be observed, and in both there is more inhibition with high concentrations than with low. (Compare Tables V and XIII.)

As with inhibition with acids and alkalis, there is less inhibition the later the heated serum is added to the mixture of unheated serum and bacteria. (Compare Tables XIV and VI.)

If bacteria be agglutinated by specific sera and the supernatant fluid removed and replaced by heated, inhibitory serum, a certain

amount of dispersion may take place or the bacteria may even be completely dispersed. Bacteria agglutinated by the group agglutinin of heterologous sera, are somewhat more readily dispersed by heated specific sera (see Table XV). This is probably due to the fact that there is much less group agglutinin, than specific agglutinin, in a specific agglutinating serum.

TABLE XV.

Rabbit *v.* typhoid serum and rabbit *v.* paratyphoid A serum. Emulsion of *B. typhosus* in saline. After 24 hours supernatant fluid removed and rabbit *v.* typhoid or rabbit *v.* paratyphoid A serum 1 : 5 in distilled water heated at 75° C. for half hour added. Contents made up to 1 cc.

Dilutions of serum in terms of original serum.

Typhoid Serum	Para. A Serum	<i>B. Typh.</i> Emulsion	After 24 hours		Typhoid Serum (75)	Para. A Serum (75)	
0.05 cc.	—	0.5 cc.	+++	{ Supernatant fluid removed and added }	0.1	—	++
0.05 cc.	—	0.5 cc.	+++		—	0.1	++
—	0.05 cc.	0.5 cc.	+++		0.1	—	+
—	0.05 cc.	0.5 cc.	+++		—	0.1	++

Different observers have obtained analogous results with precipitin anti-sera. Precipitin anti-sera are inactivated by heat and heating at high temperatures produces inhibitory properties.

The zone phenomena of heated agglutinating sera find a parallel in heated precipitin sera.

The relative inhibitory value of different heated precipitin sera varies, just as does that of heated agglutinating sera.

The time factor has the same influence with heated precipitin anti-sera as with heated agglutinating sera. Finally, as Welsh and Chapman (1909) have shown, heated precipitin anti-sera may dissolve homologous precipitate and, to some extent, heterologous precipitate also.

The interpretation of these phenomena of inactivation and inhibition is, at present, impossible. The resemblance between the effects of inactivation and inhibitory properties in heated agglutinating sera and the effects of acids and alkalis is noteworthy. The inhibitory action of heated serum is not, however, due to the production of acid or alkali in the serum, at any rate in amounts which can be demonstrated by the determination of the H-ion concentration by Sørensen's indicator method. A heated, inhibitory goat *v.* typhoid serum in dilution 1 : 10 in distilled water showed the same H-ion concentration as did the unheated serum in the same dilution.



The adherents of the Ehrlich school consider that the inhibitory action is due to the production of pro-agglutinoids and pro-precipitoids, which have a greater affinity for the antigen than have agglutinins or precipitins. The observation of Welsh and Chapman (1909) that the addition of a considerable excess of antigen did not bring the inhibition in a heated precipitin serum to an end, renders this hypothesis untenable in the precipitin reaction and probably in the agglutination reaction also.

The nature of the change in the heated serum has not been determined, but in the precipitin reaction the amount of inhibitory action seems to depend upon the amount of "precipitable substance" present in the serum and to be intimately connected with it.

Welsh and Chapman consider the fact that heating a precipitin serum to a certain temperature renders it inactive and that heating it to a higher temperature renders it inhibitory is "corroborative evidence of the conclusions, that inactivation is not due to the development of an inhibitory substance in the antiserum and that the onset of inactivation does not determine the onset of inhibition." They seem to have quite overlooked the phenomenon of "zones of inactivation."

Dreyer (1904) has shown in the case of agglutinating sera that if two sera be heated they may both show a large zone of inhibited agglutination, but in one case the titre of the serum may be much reduced, while in the other case, the serum may not be appreciably weakened.

It seems, however, much more likely that the inactivation produced by heating serum at temperatures of  $60^{\circ}$ – $72^{\circ}$  C., observed in the low dilutions is due to the formation of an inhibitory substance which, when considerably diluted, cannot produce any effect, just as was seen in the case of acids and alkalis.

The inactivation produced by heating serum to  $80^{\circ}$  C. or higher is, in all probability, due to the coagulation of the serum proteins and the removal of the agglutinins or precipitins from the solution.

Chick and Martin (1910) have pointed out that the heat coagulation of proteins—or denaturation—does not take place at any definite temperature, but is merely accelerated by raising the temperature—thus with an egg albumin solution—it took 617 minutes to reduce the amount of protein retained in solution from 9 mgm. to 3 mgm. per cubic centimetre  $69^{\circ}$  C., while it took only 7.2 minutes at  $76.3^{\circ}$  C.

It is suggestive that the inactivation and production of inhibitory properties in immune serum increase—up to a maximum—with increasing times of heating, and with increase of heat, provided the temperature is not too high. It seems possible that these effects are

due to the denaturation of a part of the serum proteins, probably those fractions which are intimately connected with the agglutinins and precipitins.

#### CONCLUSIONS.

1. The agglutination and precipitin reactions are probably essentially the same in nature.

2. The agglutination of bacteria by specific sera, is probably due to the formation of altered serum protein, in and around the bacteria and the subsequent flocculation, by electrolytes, of this altered protein and the bacteria.

3. This altered protein is probably altered serum globulin, and possibly other altered serum proteins.

4. The phenomenon of inhibition, exhibited by heated agglutinating serum, resembles closely the inhibition of agglutination by acids and alkalis.

5. The inactivation of agglutinating serum by heating at temperatures between about 60° and 72° C., and the production of "zones of inactivation," are probably due to the development of inhibitory substances and not to destruction of the agglutinin.

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FLIES AND TYPHOID.

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INTRODUCTION.

THE relation of insects to human disease has engaged a very large share of attention in recent medical research. Fleas, lice, bugs, mosquitoes and flies have severally been incriminated as carriers of infection. In every case, more or less evidence, drawn both from experiment and from observation, has been adduced to establish a case for or against, but in every instance there remains a modicum of knowledge which is still denied us. Thus, although it is an almost universal canon of scientific belief that fleas transmit plague, that certain mosquitoes transmit malaria, others yellow fever and that tsetse flies are the transmitters of sleeping sickness, yet there are numberless points on which our knowledge is by no means clear or satisfactory. It is now almost universally admitted that the common house fly, and perhaps other flies, play a certain though not extensive part in the transmission of typhoid fever. To an impartial mind it must be apparent, however, that the weight of evidence in this particular case is largely circumstantial. Although the amount of experimental work is by no means inconsiderable it must be admitted that it is not at all conclusive or convincing.

The best known and most exhaustive work on this matter is that of Graham Smith (1913). The work, however, is open to the same objection as all experimental work of the same type, namely, that it is essentially artificial and does not entirely reproduce natural conditions.

On summarising the fairly voluminous literature, as Graham Smith has done, one finds that apart from the work of that author himself there has been no extensive systematic work on the subject. Most of the contributions to the chain of evidence have been somewhat scrappy. This, however, is to a large extent in the nature of the problem and it is possible that the extensive researches of Graham Smith do not, perhaps, constitute the most important links in the chain.

#### THE ESSENTIAL POSTULATES.

It may be well to analyse the postulates necessary to incriminate the house fly as a transmitter or disseminator of typhoid fever.

It is essential that:

1. Persons infected with typhoid bacilli should provide material which may come into contact with house flies in some way or other.
2. This material should contain virulent typhoid bacilli.
3. These bacilli should be taken up by the flies from this material.
4. The bacilli should be able to exist and retain their virulence on or in the fly for a certain length of time.
5. The fly should be able to convey these bacilli to some other person or persons.
6. The bacilli when so transmitted should retain their virulence and be able to reproduce the infection.

The first postulate is satisfied by the fact that human excreta, particularly faeces, attract the common house fly both for feeding and for breeding purposes. The excreta, not only from patients suffering from typhoid fever, but also from "typhoid carriers" have been shown to contain typhoid bacilli which have not lost their virulence, a fact which satisfies the second postulate.

With regard to the third postulate the evidence is by no means conclusive. Graham Smith has shown that house flies can ingest typhoid bacilli from pure cultures and from experimentally infected material. Faichnie, alone, has shown that flies can ingest and retain *B. typhosus* from human excreta under natural conditions but his results are so remarkable that some confirmation is necessary.



The fourth and fifth postulates have by no means been satisfied. It is true that typhoid bacilli have been shown to be capable of surviving in flies for a short time when ingested in pure culture and there is no doubt that these flies can convey the bacilli to articles which furnish a ready means for further infection. It has not, however, been proved that such bacilli retain their virulence and can cause the onset of typhoid fever.

It is evident then that the weak links in the chain of evidence incriminating the house-fly as a disseminator of typhoid fever are:

1. The ability of the fly to ingest bacilli from natural sources in sufficient number.

2. Its ability to carry such bacilli living for a sufficiently long period, and

3. The ability of the bacilli to retain their virulence when deposited by the fly on materials likely to be consumed or handled by human beings.

Very little experimental work has been done towards strengthening any of these three links and it has therefore been deemed advisable to publish here the results of some experiments which may add at least a slight degree of strength to the first of them.

No mention has been made in the foregoing remarks of observational work in the field, *i.e.* in military encampments. Many observations have now been made in various campaigns and these, without exception, serve to incriminate the domestic fly as a frequent disseminator of typhoid fever: but the evidence, so derived, is, in the main, circumstantial and it yet lacks the support of careful experimental work.

The experiments about to be described were originally intended to cover the whole field of the subject, but they were interrupted at an early stage and no further opportunity of resuming the work has offered itself. It has therefore seemed desirable to publish the results in spite of their incomplete nature.

#### REVIEW OF PREVIOUS WORK.

It may be well, here, to make a few brief remarks on the observations and conclusions of Graham Smith and other workers in so far as they concern the present experiments. He remarks (1913, p. 6) "The difficulties attending the isolation and identification of pathogenic bacteria, particularly those belonging to the typhoid-colon group, from 'wild' flies are especially great, since allied, almost indistinguishable types are frequently present in the intestine of flies. No

diagnosis should therefore be accepted unless all the known tests for identification have been applied." This comment is applicable to the work of earlier observers and to that of some later workers who have not always been able, or troubled, to establish thoroughly the identity of the bacteria isolated by them.

With regard to Faichnie's work the objection is that it does not seem absolutely certain that he was actually transmitting the typhoid bacilli through the larvae to the adult flies, but was really allowing the emerging flies to contaminate themselves from the faeces to which they had access for a considerable time after emerging. The fact, however, that he submitted the bacteria recovered from the flies to independent sero diagnosis proves that he undoubtedly recovered *B. typhosus*. As will be shown presently, however, the probability is that the bacilli were not transmitted through the larvae to the adults, but were picked up by the adults themselves after emergence. The fact that he was able to recover *B. typhosus* after as long a period as 16 days is remarkable and does not accord with the experience of any other observer.

Opposed to Faichnie's assumed results are the experiments carried out by Graham Smith (1911) with the larvae of blow-flies (*Culliphora erythrocephala*) and *B. typhosus* and *B. enteritidis*. These experiments were entirely negative. Ledingham (1911) working with *Musca domestica* and *B. typhosus* had a negative experience similar to that of Graham Smith, until he adopted the plan of sterilising the external surface of the eggs and feeding the resulting larvae on large doses of pure cultures of *B. typhosus*. Under these extremely unnatural conditions typhoid bacilli were recovered from the larvae and from one pupa. Unfortunately no adult flies were hatched out. These experiments as already mentioned were extremely unnatural but were presumably the most favourable for the transmission of *B. typhosus* from larva to adult.

Ledingham also remarked on the frequent occurrence of an organism which he designates *Bacillus A* and which resembles *B. typhosus* on plate cultures, but differs from it in turning litmus milk alkaline, in producing indol and in not fermenting sorbite. Graham Smith (1913, p. 117) refers this bacillus to his type Ca 8 but in my opinion it should really be designated Ca 12 on account of the A-Alk. with reaction. A bacillus which I have frequently isolated from flies closely resembled Ledingham's bacillus A but differed in being non-motile and in fermenting sorbite slowly, approaching in these respects more closely to Graham Smith's type Ch 12, but being non-motile would correspond

to the vacant place Ch 11, or, if it fermented salicin, to Cf 11. It is to be noted that the fermentation of sorbite was slow and never occurred within 24 hours.

Nicholls (1912) has also remarked that *B. typhosus* disappeared quickly from larvae of *Sarcophaga*.

As already remarked Graham Smith (1912) was unable to recover *B. enteritidis* from blow-flies, the larvae of which had been fed on meat contaminated with that bacillus. He showed, however, that many non-lactose fermenters, such as Morgan's bacillus No. 1, will survive from the larva to the adult.

Tebbutt's results (1913) are also in opposition to those of Faichnie. He found that *B. dysenteriae* could not be recovered from pupae or adults, the larvae of which had been fed on that organism, except when the eggs were previously sterilised. *B. typhosus*, however, could not be recovered at all.

The whole subject of the transmission of bacteria from larval to adult flies is ably and briefly summed up by Graham Smith (1913, pp. 123-124) but I venture to think he is inclined to stretch a point, in endeavouring to account for the results obtained by Faichnie, by drawing the distinction, which every bacteriologist recognises, between the behaviour of cultivated and uncultivated or natural strains of the same organism. The matter, however, is one which cannot be ignored and it forms to a certain extent the *raison d'être* of the present paper. Graham Smith refers to the same subject again on p. 125 and his remarks there are worthy of the fullest consideration.

In dealing more particularly with the subject of flies and *B. typhosus*, Graham Smith (1913, p. 129) again refers to the difficulty of isolating *B. typhosus* from the intestine of the fly. His remarks are practically a paraphrase of those quoted above. He adds, however, "The occurrence of these non-lactose fermenting bacilli probably often leads to failure in isolating *B. typhosus* even when it is present, and in any case renders its detection a long and laborious undertaking." It might be added that not infrequently the reverse might be the case and organisms may be identified as *B. typhosus* which are not really so.

Ficker (1903) was able to isolate *B. typhosus* from artificially infected flies for 5-23 days after infection. In this case in establishing the identity of the organism, agglutination tests were employed. Graham Smith (1910) however, also using artificial infection, could not recover *B. typhosus* later than six days from the intestine and only two days from the faeces.

## GENERAL REMARKS ON PRESENT EXPERIMENTS.

The experimental work detailed in the following pages was carried out during my term of the Ernest Hart Memorial Scholarship of the British Medical Association (1909—1912).

Owing to the incompleteness of the results, publication has been delayed in the hope that opportunity would offer for amplification. As this hope has not been fulfilled it seems advisable to publish the results even in their incomplete state.

The experiments were started as a sequel to the work already done on the general bacteriology of *Musca domestica* (*Journal of Hygiene*, xi. 1912). The earlier work though essentially preliminary in character showed that a large variety of bacteria belonging to the colon group occurred naturally in flies. Attention was also directed to the occurrence of non-lactose-fermenting bacilli such as *B. paratyphosus* B and Morgan's bacillus No. 1 in flies in the natural state. No mention, however, was made of a large number of other non-lactose-fermenting bacteria which were isolated and carefully studied as it was impossible to identify them with any known pathogenic or non-pathogenic forms. Their characters presented many difficulties with which, at that time, I did not feel prepared to cope. Since then several authors, particularly Graham Smith (1912), have published descriptions of non-lactose-fermenting bacilli, agreeing closely with those met with during the course of my investigations.

Quite a number of the organisms referred to above initially bore a striking resemblance to *B. typhosus*. On neutral red bile salt media they were indistinguishable from colonies of *B. typhosus* except that they appeared a trifle brownish after 24 hours incubation at 37° C. It was discovered later however that the same colonies would appear when the plates were incubated at room temperature (15°—20° C.). Hundreds of these colonies were sub-cultured in fermentable media and in a large number of cases gave, for the first few days, reactions identical with that of *B. typhosus*. In every case agglutination tests were made with stock typhoid serum and in the large majority of cases the result was unequivocally negative. In the few doubtful cases the agglutination tests were repeated and absorption tests were also employed. For help in these tests I am indebted to Drs Bainbridge, Ledingham and Petrie, who made independent controls.

In every case the results were negative and this was confirmed by the fact that on the fourth to the sixth day the cultures began to



ferment lactose. By the sixth day in the great majority of cases the lactose broth showed a distinct reddish colour and in the few other cases the appearance of the red colour was not delayed beyond the tenth day.

The foregoing remarks apply only to organisms apparently resembling those of the typhoid group. Numerous other non-lactose fermenters were isolated resembling essentially *B. paratyphosus* B and Morgan's bacillus No. 1 but differing from them in one or more important details. It is worthy of remark here that no organisms resembling *B. paratyphosus* A or *B. enteritidis* Gaertner were ever isolated from the flies I examined.

It should be noted that the preliminary methods of identification employed in these investigations were those adopted by Morgan and Ledingham (1909) and by Graham Smith (1912).

The bacilli mentioned in the foregoing paragraphs will be dealt with in more detail in the second part of this paper. The more immediate object of the present communication is to discuss the results of experiments in which there could be no question that *B. typhosus* was actually involved.

The particular experiments on *B. typhosus* were commenced in June, 1911, and continued till February, 1912. The flies used were entirely *Musca domestica*, the majority being flies reared from larvae in captivity, the others being "wild" flies captured in the warmer parts of the Institute. It may be remarked that no flies were taken from any quarter within a radius of 80 yards of any place where typhoid experiments were being undertaken. As a matter of fact the only two places in which *B. typhosus* was present were the laboratory of the chief bacteriologist and the stock culture room, both more than 20 yards from the experimental room and neither likely to contain exposed cultures of *B. typhosus*. The infective material was obtained from Dr Ledingham, Chief Bacteriologist at the Lister Institute, and consisted of the faeces of suspected typhoid carriers, which Dr Ledingham was systematically examining for the Local Government Board. In every case the faeces sent up to me were from cases which had proved to be undoubted typhoid carriers, but the particular sample submitted to me was not guaranteed to contain *B. typhosus*.

On the material thus obtained batches of flies were fed for periods varying from 20 to 60 minutes. From the material cultures were made and found to contain *B. typhosus* in every case except two. These findings were later confirmed by the report from the laboratory of the chief bacteriologist.

After the flies had been allowed to feed on the infected material, it was removed and they remained for a longer or shorter period without food. The members of each batch of flies were examined over a period of 48 hours. Each batch of flies consisted of about half a dozen, which from previous experience had been found the most convenient number to deal with. In the first seven batches of flies the results, so far as *B. typhosus* was concerned, were entirely negative. In the eighth series of seven flies one was discovered to harbour *B. typhosus* 28 hours after feeding. The next five batches were entirely negative. The fifteenth showed one fly infected after 5 hours and another after 14 hours. The next three batches were negative. The nineteenth gave the most favourable result. One fly showed infection 45 hours after feeding while another gave a culture 36 hours after feeding. Of the remaining seven batches only two were positive and both under 24 hours. One gave a positive result in 23 hours and the other in 9 hours. Thus out of a total number of 163 flies used only 7 gave a positive result.

It is thus evident that the chance of flies becoming infected from infective faeces under these experimental conditions is not very large and under natural conditions the chance must be very considerably less.

The experiments were conducted for the most part in sterilised glass chimneys, closed at the top end by a layer of muslin and at the bottom by a glass plate. Several, however, were conducted in a specially constructed cage described in a previous paper (Nicoll, 1912). Each batch consisted of 7 to 8 flies which were allowed to feed on the infected faeces for a period of from 4 to 24 hours. The faeces was then replaced by sterilised sugar and water. A 1 per cent. solution of cane sugar in distilled water was used. After the lapse of about an hour one fly was removed and examined. The procedure was similar to that already described (Nicoll, 1912). The fly was first thoroughly washed in peptone broth which was thereafter incubated at 37° C. It was then washed in sterile water and then immersed for 10 to 20 minutes in absolute alcohol. After being dried over a flame it was again washed in broth which was incubated as a control. The fly was then dissected and the alimentary canal teased up in broth and incubated. In several cases the various parts of the fly were incubated in separate tubes of broth.

The broths were incubated for 12 to 18 hours, then plated on neutralised bile salt agar. From these plates all white colonies were picked off, or in cases showing an excessive number of white colonies about

ten or a dozen were selected. Each colony was again incubated in peptone broth and replated. The pure cultures so obtained were submitted to the usual tests.

The remaining flies of each batch were examined at intervals, and at the conclusion of the experiment cultures were made from the sugar and water and from the vomit and faecal marks left on the glass chimneys and plates.

The tests used in identification were the fermentative reactions in lactose, glucose, mannitol, saccharose, dulcitol, adonitol, sorbitol and litmus milk together with the motility, production of indol and agglutination with stock typhoid serum.

A fact which is probably of little significance, but none the less worthy of note is that there was a comparatively high mortality amongst the flies used in the experiments. This was most noticeable in the winter months (December to February) but was also remarked on during the summer and autumn while other experiments, in which flies were fed on faeces from various sources, were being conducted. It may be a matter of mere coincidence, but the observation is suggestive of the possibility that these faeces contained something deleterious to the flies.

In the course of the experiments 1062 colonies were examined, 578 of those proved to be colonies having little in common with the typhoid and allied groups of bacilli. Of the remaining 484 colonies, 361 did not ferment mannitol. There remained a total of 123 which appeared to have some relation to *B. typhosus*: 98 of those, however, were non-motile organisms which eventually fermented lactose (in 4 to 6 days). Thus only 25 of the 1062 colonies proved to be *B. typhosus*, and these as already mentioned were obtained from 7 flies.

As was to be expected, in most cases more than one colony of the same type was picked off from one plate. These duplicates except in the case of *B. typhosus* were discarded and there remained 357 separate cultures. 25 of these, as already mentioned, were *B. typhosus*. The other 332 cultures were found to comprise 49 different varieties of bacilli. On an average, therefore, on every fly examined there were at least two varieties of non-lactose-fermenting bacilli. As mentioned above a considerable percentage of these proved to be in reality slow lactose fermenters and they caused considerable trouble in the course of the investigations.

Throughout the course of the experiments four days was taken as the critical period for the fermentative reactions though actually the

period was  $3\frac{1}{2}$  days, as the media were usually incubated in the afternoon and examined on the morning of the fourth day. As a matter of fact in the vast majority of cases constant results were obtained on the morning of the second day (*i.e.* after about 42 hours). In every case, however, the media were kept up till the tenth day, but changes after the sixth day (morning) were neglected: except in the case of litmus milk which was kept for fifteen days.

Reference has already been made to the frequent presence of bacilli resembling *B. typhosus* in all their fermentative reactions, and the indol reaction. The action in litmus milk too, as shown later, was identical yet the organisms were invariably non-motile and did not agglutinate with typhoid serum. The investigation of these bacilli consumed a considerable amount of time and resulted in the conclusion that organisms of this type flourish in flies. This observation finds confirmation in the work of Ledingham and Graham Smith who frequently found bacilli identical with those met with in my experiments. These independent findings cannot fail to raise the question of the occurrence of a specific bacterial flora in the intestine of the house fly. I have frequently obtained almost pure cultures of bacilli of this type from the intestine of flies, and the fact causes one to pause in estimating the results of earlier investigations. It cannot be doubted that many of these organisms might readily be mistaken for *B. typhosus*, and even such an experienced bacteriologist as Graham Smith expressed his uncertainty when he first encountered them.

It is a curious fact too that Ledingham in the course of his massive-infection experiments with fly larvae and *B. typhosus* cultures should have encountered this type of bacillus so frequently. These results from well accredited sources throw very great doubt on the experimental results of Bahr. On the other hand it might be assumed that Bahr is correct and that bacteriologists such as Ledingham and Graham Smith have not grasped the true facts of the case, namely that *B. typhosus* and other organisms have been transformed in the intestine of the fly or its larvae into bacilli of a somewhat different type. On this matter or indeed on the transmissibility of any species of organism, be it bacteria, plant or animal, I have an open mind but my results in the present instance endorse the caution of Graham Smith and the almost uncompromising negativeness of Ledingham.

As an addendum to the foregoing it may be remembered that I have conducted experiments with a view to determining what might be considered the natural intestinal flora of flies. These experiments



were unfortunately not entirely satisfactory as a number of discrepant results were obtained. On the whole, however, they showed several interesting facts. Batches of "wild" flies were captured and enclosed in glass chimneys. They were fed on sterile foods for various periods. Two or three of each batch were examined bacteriologically at the outset and were found to contain an almost overwhelming preponderance of organisms of the *B. coli* type. The remaining flies were fed on sterile food, chiefly water, sugar and bread. It was found that after a week the *colon* bacilli had very largely disappeared. After 13 to 14 days *colon* bacilli had absolutely disappeared, except in a few cases. After the eighth day, as a rule, the bacterial flora became almost purely non-lactose or apparently so. There was an absolute absence of bacilli of the typhoid-colon type. After the eighth day the bacterial flora began to display a characteristic non-lactose type. These bacteria were mainly of the type already mentioned above and were in reality not non-lactose fermenters in the strict sense of the term. In addition, however, a considerable number were met with conforming to the type of Morgan's No. 1 bacillus.

With regard to the feeding of flies many observations have already been made. Graham Smith has given a very full and complete account of this matter, and I have added one or two observations which may serve to show that flies are attracted by excrement both from human and other sources. It was pointed out in the previous paper that an essential point was that the excrement should be fresh and moist. When it becomes dry after the lapse of several hours the attraction is very greatly diminished. On that account, in the foregoing experiments it was necessary to moisten the material every 24 hours to start with and eventually twice and three times a day. Naturally after the first 24 hours the freshness had gone and it was only by the added moisture that the flies could be attracted.

From the foregoing paragraph it is evident that flies are attracted chiefly by moist excreta and this, as far as the dispersal of pathogenic bacteria is concerned, seems to be an argument against the disposal of human faeces in shady, sheltered places where they remain moist and fresh for a considerably longer period than if they were exposed to the open air or the direct rays of the sun. From these observations it is obvious that the ordinary field-latrine, dug in a secluded place if possible, is one of the most favourable feeding and breeding grounds for flies. It is, as a rule, sufficiently deep to exclude the sun's direct rays, and from the accumulated urine it is kept in a sufficiently moist

state to attract flies. It is true instructions are given for the use of sawdust, sand and other absorbent materials, but the chance of those being employed as a routine is extremely remote. This view is derived from experience in civilian centres where a very large percentage of the population ignore such precautionary measures.

#### DETAILS OF EXPERIMENTS.

- |    |          |        |  |   |
|----|----------|--------|--|---|
| I. | June 16. | 6 p.m. | Six flies enclosed in one section of experimental cage along with normal human faeces impregnated with urine from typhoid patient. |   |
|    | „        | 16.    | 7.30 p.m.  | Three flies transferred into second section of experimental cage in which some sterile moist bread had been placed.   |
|    | „        | 17.    | 3.30 p.m.  | Bread removed and cultures made (Culture No. 1). Fresh bread introduced.  |
|    | „        | 17.    | 4 p.m.   | One fly from Section II examined. After immersion in absolute alcohol and normal saline, washed in peptone broth (Culture 2). Cultures also made from the intestines (No. 3), crop (4) and salivary glands (5). |
|    | „        | 18.    | 4 p.m.   | Cultures made from bread (6); fresh bread introduced.   |
|    | „        | 19.    | 8 p.m.   | Cultures made from bread (7). Several flies dissected, cultures made from alimentary canal (8).   |
|    | „        | 21.    | 9 a.m.   | Remaining flies dead. Combined cultures made from their alimentary canal (9).   |

All these broth cultures were plated on neutral-red bile salt agar, and it was found that Nos. 2 and 8 were entirely sterile, while Nos. 1, 3, 4, 5, 6 and 7 showed only red colonies. No. 9 showed numerous white colonies which, however, were not *B. typhosus*. No red colonies were present.

- |     |          |          |  |   |
|-----|----------|----------|--|---|
| II. | June 22. | 12 noon. | Twenty flies put into cage along with faeces from typhoid patient. |   |
|     | „        | 22.      | 7 p.m.   | Six flies transferred to second section of cage into which solution of sugar in water introduced (majority of flies in Section I already dead).   |
|     | „        | 23.      | 12 noon.   | Two flies dead in Section II; others living. Cultures made as follows:—exterior of first fly (10) exterior (11) and elementary canal (12) of 2nd fly; also from intestine of fly in Section I (13) and from sugar solution (14). Fresh water and bread introduced Section II. |
|     | „        | 24.      | 4 p.m.   | Bread removed from Section II. Cultures made from it into broth (15) and directly on to bile salt agar (16). All flies in Section II living: only three living in Section I. Cultures made from fly from Section II; external (17) internal, intestine (18) and crop (19).    |

June 27. 4 p.m. Cultures made from faeces:—1. Direct on to bile salt agar (20). 2. Into peptone broth (21). Bread removed from Section II and cultures made. Broth cultures (22) and direct plate (23). Emulsion made from dead flies in Section I, cultures (24 and 25): plate (26).

The results of these cultures were as follows:—

No. 10. Showed only cocci.

No. 11. Had cocci and colon bacilli.

Nos. 12 and 13. Only coli.

No. 14. Sterile.

No. 15. Coli, cocci and one white colony which was not *B. typhosus*.

No. 16. Coli, cocci and some white colonies which were not *B. typhosus*.

No. 17. Cocci only.

No. 18. Coli and cocci. No white colonies.

No. 19. Sterile.

No. 20. Only coli.

No. 21. Coli and white colonies which produced gas in mannite.

No. 22. No white colonies.

Nos. 23, 24 and 25. Several white colonies which produced gas in mannite.

No. 26. Coli and several white colonies resembling *B. typhosus* but differing in several reactions.

From this it can be seen that none of the cultures made in this experiment contained *B. typhosus*. Cultures made from the infected material at the end of the experiment proved to be negative which might be taken as evidence that the typhoid bacilli had all died out.

III. June 27. 5 p.m. Fifteen flies introduced into cage with infected faeces from typhoid carrier, cultures made from faeces (27, 28) moist bread placed in Section I.

„ 28. 10 a.m. Six flies transferred to Section II into which a piece of moist bread was introduced.

„ 28. 6 p.m. Cultures made from bread in Section I (29).

„ 30. 1 p.m. Cultures again made from bread in Section I, Plate (30) Broth (31). Then flies from Section II examined together. Cultures made from external surface (32) and alimentary canal (33).

„ 30. 6 p.m. Cultures made from fly faeces on bread dish in Section I, plate (34) and broth (35).

July 1. 7 p.m. Three flies from Section II examined. Cultures from exterior (36), alimentary canal (37). Cultures made from bread in Section II (38). Cultures also made from faeces (39). Experiment discontinued.

The results of these cultures were

The cultures from the faeces on 27th June showed the presence of *B. typhosus*, but on 1st July the cultures were negative. From the flies only *B. coli* and cocci were obtained. The bread showed the same organisms, while the cultures made from the fly faeces proved sterile.

IV. July 5. 4 p.m.	Thirty flies put into Section I of cage along with moist faeces from typhoid carrier and some fresh bread.
„ 6. 4 p.m.	Fourteen flies transferred to Section II. Cultures made from one fly, exterior (40) and alimentary canal (41).
„ 7. 11 a.m.	Plate culture made from faeces (42) from bread in Section I (43) and from bread in Section II (44). Four flies from Section II examined, and cultures made from exterior and alimentary canal (45—52).
„ 8. 4 p.m.	Ten flies from Section II examined. Cultures 53, 54, 56 and 57. Cultures made from bread in Section II (55) and from bread in Section I (58).

This experiment was more interesting than the previous three owing to the fact that a large proportion of the cultures showed the presence of non-lactose-fermenting organisms. The plate cultures made from the typhoid faeces displayed only coliform colonies. The alimentary canal of the flies provided in every case almost pure cultures of non-lactose-fermenting bacilli, all of which either did not ferment mannite or produced gas on that medium. The exterior of the flies provided a more varied flora of colon bacilli, cocci and occasional non-lactose bacilli. The cultures from the bread showed in every case non-lactose bacilli which fermented mannite with gas production. *B. typhosus* was not isolated in any case.

V. On July 9th a fresh sample of faeces from a typhoid carrier was placed in the cage along with the flies left over from the foregoing experiment. On the 9th fifteen flies were transferred from Section I to II. Five hours later four of these flies were dissected and cultures were made from their external surface and their alimentary canal. In every case non-lactose organisms were isolated from the intestine and in one case from the external surface. In three instances, the organisms so isolated did not ferment mannite, while the other two fermented it with gas formation. Twenty-four hours later another three flies were examined with similar results. The following day (July 11th) four flies were examined *en masse*. From the external surface several non-lactose colonies were isolated and these either did not ferment mannite or produced gas on that medium. From the intestine numerous non-lactose colonies were isolated. The majority of these produced gas on mannite but a few fermented that medium without the production of gas. In many other respects these organisms resembled *B. typhosus* but they fermented saccharose. Several cultures were tested with typhoid serum but all failed to agglutinate. A few hours later another two flies were examined in a similar fashion and gave results identical with the previous batch. On the same day two lots of five flies each, from Section I, were examined. In both cases almost pure cultures of non-lactose-fermenting organisms were isolated, but these proved to be either non-mannite or mannite fermenters with gas production. On July 12th another batch of four flies from Section I was examined and gave results similar to the previous lots. Two days later a further lot of four flies from Section I was examined. From the external surface of these an almost pure culture of non-lactose organisms producing gas on mannite was obtained, while from the intestine a pure culture of non-lactose, non-mannite colonies was obtained.



At the same time two flies from Section II were examined with similar results. Two days later another two flies from Section I were examined and again both results were similar to the foregoing. Three days later the experiment was concluded. Ten flies were left in Section I. These were divided into three lots of two, four and four respectively. From the external surface of the first lot several lactose-fermenting organisms were obtained, while from the intestine an almost pure culture of a non-lactose, mannite fermenting bacillus was obtained. This bacillus, however, fermented saccharose and liquefied gelatine. From the second lot an almost pure culture of non-lactose, non-mannite bacilli was obtained. These organisms fermented glucose with gas formation. From the last lot the organisms isolated were chiefly non-lactose with gas on mannite.

This experiment is interesting in several respects. As far as *B. typhosus* is concerned it was entirely negative, of that there is no question. From start to finish the last batch of flies was in captivity for a fortnight. The time of the year—the middle of summer—was that most favourable for the life of flies and as has been stated in a report on previous experiments flies can be kept alive and in healthy condition in captivity for at least six weeks. My endeavour throughout the experiment was to keep the flies in a healthy condition.

Fresh water passed through a Berkefeld filter and moist bread, boiled in water for ten minutes, were supplied to them every day or as occasion required. The most remarkable result is the disappearance of the lactose-fermenting organisms of the *B. coli* type and their replacement by non-lactose-fermenting organisms. This curious phenomenon has been referred to in the paper mentioned above. These organisms fell naturally into three groups according to their behaviour on mannite, namely non-fermentation, fermentation without gas production and fermentation with gas. In the first category the organisms were mainly of the type of Morgan Nos. 1 and 2, though a few were found corresponding to type V. In the second category three of the types (Flexner) designated by Morgan and Ledingham (1909) occurred along with a few others not included in their list. In the third category the majority of the organisms isolated conformed to type No. X of the above classification, but a few were also found of the type XII and XIV.

VI—VIII. During September three further sets of experiments were conducted on the same lines. In each case the results were entirely negative.

IX—XI. During October another three sets of experiments were carried out. At this time the flies began to be attacked by the fungus, *Empusa muscae*, and the mortality from this cause was 30 to 40 per cent. The technique in these experiments was slightly modified. The various parts of the flies were lightly mashed up in broth and placed in the incubator at 37° C. for four hours. Thereafter the emulsions were thoroughly ground up and cultures made in broth or direct on bile salt agar. Forty-seven flies were used in these experiments and in every case the result was negative.

XII. On November 1st a different experiment was tried. A culture of *B. typhosus* was made from the faeces of a typhoid carrier (C. R.) and a sub-culture of this was made on plain agar. From this an emulsion was prepared and placed along with some sugar in a vessel into which five healthy flies were introduced. Seven hours later two flies were examined and from their legs a pure culture of typhoid bacilli

was obtained. This was identified by all the possible tests except inoculation. From the intestine and crop three different white colonies were obtained. One of these resembled *B. typhosus* except that it was non-motile and produced no reaction on litmus milk. Of the other two varieties one gave an alkaline reaction on milk and produced indol, while the other fermented saccharose and was non-motile. None of these strains would agglutinate with typhoid serum even in low dilution. Cultures were made from the faecal marks left by the flies. Here again white colonies were obtained but they were non-motile and fermented lactose after six days. Cultures from the vomit marks were negative.

The flies were then transferred to a clean vessel and after 24 hours the faecal and vomit deposits were again cultured with negative results. On November 2nd the flies were again transferred to a third vessel and allowed to remain in it for nearly ten days. At the end of 43 hours cultures were made from the faecal deposits which were negative. Cultures from the vomit marks, which were almost solid deposits of sugar, gave a growth of almost pure *B. typhosus*. The bacilli gave an agglutination of 1 in 2000 with typhoid serum.

Five hours later a single fly from this batch was examined: the legs and proboscis gave a negative result but from the intestine several colonies were isolated which proved to be *B. typhosus*, giving an agglutination of 1 in 2000 with typhoid serum. The remaining flies of this batch were examined, one every day, but gave a consistently negative result. The faecal and vomit deposits were also examined but they, too, were negative.

XIII. On November 2nd six flies were placed in a vessel with faeces from a typhoid carrier. They were allowed to remain undisturbed till November 5th when one fly was examined. Cultures from its legs gave only *B. coli*. From the intestine numerous white colonies were obtained, but they all proved to be slow lactose fermenters. Two days later another fly was examined, and both from the external surface and from the intestine white colonies were obtained. In every case, however, they were non-motile organisms and did not ferment mannite. On the following day the last living fly was examined. From its legs only cultures of cocci were obtained but from its intestine numerous colonies of a motile bacillus were obtained. From the sugar reactions these organisms appeared to be *B. typhosus* but they produced indol, and on litmus milk they gave, at first, an acid reaction but eventually an alkaline one. On being tested with typhoid serum there was no agglutination.

XIV. On November 4th six flies were placed in a vessel with faeces from the same typhoid carrier. Five hours later cultures were made from the faeces. No typhoid bacilli were obtained. All the organisms isolated were colon bacilli. At the same time a single fly was examined. Nothing of note was found on the legs or exterior but from the intestine numerous non-lactose colonies were obtained. On further investigation these were identified as Morgan's bacillus No. 1. Next day another fly was examined. From its legs an almost pure culture of a non-lactose-fermenting organism was obtained. This resembled Morgan's bacillus, but its identity was not absolutely established. From the intestine, on the other hand, a pure culture of a non-lactose-fermenting bacillus was obtained. This resembled *B. typhosus* in all its reactions except on litmus milk which it turned

from acid to alkaline on the third day. On being tested with typhoid serum it gave a negative result (1/1000 with a serum of high titre). Two days later another fly was examined. Cultures from its legs were sterile. From the intestine numerous colonies of a slow lactose-fermenting bacillus were obtained. This was motile and fermented glucose, mannite and saccharose slowly. It produced no indol and turned litmus milk permanently acid, but produced no clot. Several non-mannite colonies were also isolated.

On November 8th another fly was examined. From its legs an almost pure culture of *B. paratyphosus* B was obtained. This organism agreed in all its cultural reactions with those of *B. paratyphosus* B except that it produced very little gas on mannite. It agglutinated with paratyphoid serum up to 1 in 500. From the intestine and crop of the same fly cultures were made but these proved sterile.

On November 9th another fly was examined, from its external surface nothing but non-lactose colonies were plated, these proved to be Morgan's No. 1 bacillus.

On November 10th another fly was examined. The external surface yielded no growth and the intestine gave only cocci.

On November 11th the last fly of this batch was killed and examined. From the legs and the intestine almost pure cultures of a non-lactose organism were obtained. These again proved to be identical with *B. paratyphosus* B and they agglutinated with paratyphoid serum to 1 in 500.

XV. On November 7th three flies were placed along with faeces from a typhoid carrier (J. C. 5). Cultures were made from the faeces but no *B. typhosus* were isolated. Eighteen hours later the faeces were found to be dried up and a fresh lot was introduced. Four hours later one fly was examined. From the external surface nothing was obtained, but from the intestine an organism closely resembling *B. typhosus* was obtained in large numbers. It produced indol, however, and gave an acid to alkaline reaction on litmus milk. It probably corresponds to Morgan and Ledingham's bacillus No. 4 B. At the same time cultures were made from the faecal and vomit marks made by the flies. Neither of these yielded anything.

On the 9th a second fly was examined. The external surface was quite sterile and the intestine yielded only a few cocci and lactose fermenters.

On November 12th the last fly was examined and yielded only lactose fermenters both internally and externally. The faecal marks from the vessel were examined but proved negative.

XVI. On November 7th five flies were put along with faeces from a typhoid carrier (C. R. 7. XI). Twenty hours later one fly was examined. From the external surface only cocci were obtained. From the intestine a bacillus was obtained which was motile, did not ferment lactose on mannite but produced acid and gas on glucose and saccharose. It produced indol and turned litmus milk slightly acid. A second fly gave only lactose fermenters and cocci.

Next day a third fly was examined. From the legs only lactose fermenters were isolated. From the intestine a few non-lactose colonies giving acid and gas on mannite were isolated but these were not further identified. From the faecal marks some non-mannite colonies were isolated but not further examined, while from the vomit marks nothing was obtained.



On the 10th another fly produced only cocci both externally and internally. The faecal and vomit marks were sterile. Two days later a dead fly was examined. From the external surface an almost pure culture of a non-lactose bacillus was obtained. This gave acid and gas on glucose and mannite, was non-motile and did not produce indol. The milk reaction was acid to alkaline. From the intestine a different type was isolated. This resembled Morgan and Ledingham's No. 3, except that it turned litmus milk from slightly acid to slightly alkaline. The change, however, was so very slight that it might reasonably be ignored.

On November 14th the remaining flies were dead. One was examined. From the legs no growth was obtained and from the intestine only lactose fermenters were isolated.

XVII. On November 10th five flies were placed along with some faeces (Macconachie 5. XI.). Seven hours later one fly was examined. In the cultures from the legs all the colonies were lactose fermenters. From the intestine a number of slow lactose fermenters were obtained. These fermented mannite and glucose with gas production but did not affect saccharose or dulseite. They gave an alkaline reaction on litmus milk, later turning slightly acid. They were not further investigated.

On November 11th another fly was examined. From its external surface a number of apparently non-lactose organisms were isolated. They fermented both glucose and mannite and after five days they gave a slight acid reaction on lactose and saccharose. On litmus milk they gave a distinct acid reaction all through. From the intestine a large number of white colonies were obtained but none of them fermented mannite and they were accordingly discarded. The faecal and vomit marks were examined. From the former only lactose fermenters were obtained while the latter was sterile.

Next day another fly yielded, on its legs, only lactose-fermenting colonies while the intestine produced white colonies which fermented mannite and glucose with gas production, were non-motile, produced no indol and gave an alkaline reaction on litmus milk.

Ten days later cultures were made from the faecal and vomit marks, both of which were sterile.

On November 16th a third fly was examined, its legs proved to be sterile, but in the intestine an almost pure culture of a non-lactose fermenter was present. This gave acid and gas on mannite, glucose and saccharose, was motile, did not produce indol and gave an acid to alkaline reaction on litmus milk.

Three days later (Nov. 19th) another fly was examined. From its legs only cocci were obtained while from the intestine a single red colony was obtained. Four days later (22nd) the last fly, which was still living, was examined. Neither from its legs nor intestine were any organisms obtained.

XVIII. On November 20th a six hours' broth culture was made from the faeces of a typhoid carrier (Simpson). Four flies were allowed to feed on this. Five hours later one fly had fallen into the culture. It was killed and examined. The external cultures were discarded. From the intestine a large number of white colonies were isolated. On further examination these proved to be *B. typhosus*. The serum reaction confirmed the diagnosis. Next day another fly was examined. From its legs several non-lactose fermenters were isolated. These differed from *B. typhosus*



only in the fact that they produced indol and turned litmus milk alkaline. From the intestine a single red colony was obtained, together with several whites. The latter were non-motile and produced acid and gas on glucose, mannite and saccharose. They produced indol and on litmus milk gave an acid to alkaline reaction. From the vomit deposits pure cultures of *B. typhosus* were obtained.

Three days later the vomit marks were again examined, but proved sterile. After another two days the vomit and the faecal marks were examined but proved negative. Three days later all the flies were dead, one was examined but produced only cocci both internally and externally. The other flies were not examined.

From these foregoing eighteen experiments it is evident that the task of isolating *B. typhosus* from flies exposed to contamination is by no means an easy one. Only in the last experiment where flies were allowed access to a pure broth culture were typhoid bacilli obtained from the flies. In almost every other case non-lactose fermenters were isolated, some of which appeared to resemble *B. typhosus* but on careful examination were found to be distinctly different organisms. Again a number of the organisms isolated appeared to be identical with *B. paratyphosus* B and Morgan's bacillus No. 1, both of which are regarded as pathogenic in some degree.

The other non-lactose colonies isolated were referable to some extent to organisms isolated by Morgan and Ledingham and by Graham Smith, but a number appeared to be different to any which these authors succeeded in isolating. Again there were a large number of slow lactose-fermenting organisms which closely resembled *B. typhosus* except in their inability to agglutinate with typhoid serum. As far as I can gather these have not previously been referred to by workers on this subject.

XIX. On November 29th four flies were placed along with faeces from Mrs Brassell (25. xi. 12). Twenty-six hours later the faeces were removed. One fly was killed and examined. From the external surface a number of non-lactose-fermenting organisms were isolated none of which proved to be *B. typhosus*. From the alimentary canal, however, a large growth of non-lactose-fermenting colonies were obtained. The crop and the intestine were examined separately. From the former an almost pure culture of *B. typhosus* was obtained. These organisms gave all the typical cultural reactions of *B. typhosus* and in addition they agglutinated with typhoid serum up to 1 in 2000. From the intestine numerous colonies of *B. typhosus* were isolated. Along with them, however, were several other non-lactose colonies. Most of these resembled *B. typhosus* except that they were non-motile. The others differed in not fermenting sorbite and in turning litmus milk from acid to alkaline. The faecal matter from this batch of flies was examined and found to contain *B. typhosus* together with other non-lactose fermenters which fermented glucose, mannite and saccharose with gas production but did not ferment sorbite. They were motile but gave no indol. They probably corresponded to Morgan and Ledingham's type XII 12, as their litmus milk reaction was A.A.A.C. From the vomit marks nothing was obtained.

After another twenty-six hours a second fly was examined. Cultures from its legs produced a few white colonies which were not *B. typhosus*. After being thoroughly sterilised the fly was mashed up in sterile broth and plates made directly from the emulsion. On these several colonies of *B. typhosus* were found, these gave a positive agglutination up to 1 in 2000. The faecal and vomit marks from the

flies were again examined. The former yielded only a few lactose fermenters, while the latter was negative. Next day the third fly was examined. From its legs numerous non-lactose colonies were obtained which proved to be identical with Morgan and Ledingham's type 4 B. From the intestine several non-mannite, non-glucose bacilli were isolated, but were not further identified. *B. typhosus* was not recovered. The last fly of this batch was found dead next day and as it was dried up it was not examined.

XX. On November 30th nine flies were placed along with faeces from C. R. (29. XI.). Twenty-five hours later the faeces were removed. From the legs of one fly numerous non-lactose colonies were isolated. These proved to be identical with type 4 B (Morgan and Ledingham) in all their cultural reactions. From the intestine only some non-mannite fermenters were isolated. From the faecal marks left by the flies *Bacillus* 4 B was again isolated in considerable numbers. The vomit marks yielded only non-mannite fermenters. A second fly examined twenty-four hours later yielded an exactly similar result, *Bacillus* 4 B being found on the legs and in the faecal deposits. The same organism was isolated thirty hours later from a third fly, this time both from the legs and the intestine. Two days later all the flies were dead. They were examined in two batches. From the legs of the first an almost pure culture of an organism resembling type 3 (Morgan and Ledingham) was obtained. These fermented lactose slightly after six days. From the intestine nothing of interest was obtained. From the legs of the second batch what was probably an organism identical with that just mentioned was obtained. The intestine again yielded nothing of interest.

XXI. On December 5th ten flies were put along with faeces from Mrs B. which were heavily charged with *B. typhosus*. Twenty-five hours later the faeces were removed and sugar solutions substituted. By this time two flies had already died. One living fly was examined but was accidentally immersed in chloroform. No growth was obtained from it. Twenty-three hours later four of the remaining flies were dead and one was dying. These were all examined. From the external surface only a few non-lactose colonies were obtained. These proved to be chiefly non-mannite fermenters with a few which produced acid and gas on mannite. From the intestine only lactose-fermenting organisms were obtained. The faecal and vomit marks were examined. The former yielded only cocci and one coliform bacillus, while the latter gave nothing.

The remaining two flies were transferred to a fresh vessel. Two days later one was examined, but yielded only a few non-mannite bacilli both externally and internally. Three days later the last fly was examined. Only a few cocci were obtained from it.

XXII. On December 13th eight flies were placed with faeces from C. R. On examination no *B. typhosus* could be found in the faeces, but there was a plentiful growth of a bacillus which produced white colonies on MacConkey's medium greatly resembling that of *B. typhosus*. Its cultural reactions resembled those of type 3 (Morgan and Ledingham). It produced acid on mannite, glucose and sorbite but not on saccharose. It was non-motile and gave a strong indol reaction. On litmus milk it gave an acid to alkaline reaction and on lactose it gave a faintly acid reaction after six days.

Four hours later the faeces were removed and replaced by sugar and water. On examination one fly yielded the above described bacillus in almost pure culture both on legs and in intestine. A second fly yielded the same bacillus from its intestine but no growth was obtained from its legs. From a third fly the same bacillus was removed in almost pure culture from legs, crop and intestine.

Next day two flies yielded the same bacillus in great abundance both on the legs and in the alimentary canal. Twenty-four hours later another fly gave a growth of the same bacillus from its intestine but on its legs nothing but coliform organisms were found. A day later another fly yielded the same bacillus both internally and externally with an addition of a few non-mannite fermenters on its legs. Five days later the last fly gave a culture of the same bacillus together with numerous cocci from its intestine, but no growth was obtained from its legs.

XXIII. On December 15th five flies were put with faeces from Mrs B., from which typhoid bacilli were recovered. Four hours later the faeces were removed and replaced by sugar and water. One fly was examined but the plates were so thickly overgrown that no organisms could be isolated. Two hours later a second fly yielded a number of non-mannite colonies from its intestine but no growth from its legs. Next day a third fly gave no growth from its legs or intestine. Two days later the last two flies were killed. From their legs only cocci were recovered while from the intestine there was a growth of cocci and non-mannite fermenters.

XXIV. This experiment was conducted with normal faeces which were inoculated with a twenty hours' broth culture of typhoid bacilli. Only three flies were used; they were allowed access to the faeces for two days at the end of which time they were removed. One fly was then examined. Cultures were only made from the intestine. All the organisms obtained were non-lactose fermenters but *B. typhosus* was not recovered. The chief organism isolated was one that resembled typhoid except that it did not ferment sorbite, produced indol and discoloured litmus milk. A day later the same organism was recovered in large numbers, but always with it a few typhoid colonies were isolated. Two days later a similar result was obtained with the third fly.

#### SUMMARY AND CONCLUSIONS.

1. The chain of evidence incriminating the house fly as a disseminator of typhoid fever is at present fairly complete, but many of the links are weak and not thoroughly strengthened by experimentation.

2. The bulk of experimental work has hitherto been done under highly unnatural and artificial circumstances and the results so obtained cannot be accepted unreservedly as giving a correct view of conditions in nature.

3. The experiments described in the present paper show that flies can ingest typhoid bacilli from natural matter, *i.e.* human faeces and urine, and carry them for a certain period of time.

4. There is no evidence to show that the typhoid bacilli multiply in the house fly. On the contrary the evidence goes to show that they are not adapted for prolonged life on or in the fly.



5. It thus follows that the house fly is a purely mechanical carrier of the typhoid bacillus and is not a natural "host" in the strict sense of the term.

6. Many bacilli closely resembling *B. typhosus* in cultural characteristics appear to be natural or, at least, common inhabitants of the intestine of the house fly. These are extremely likely to be mistaken for *B. typhosus* unless the most stringent tests are employed.

7. As might be expected there is evidence to show that a process of bacterial selection occurs in the fly's intestine. Some bacteria appear to flourish but others are rapidly eliminated. Among the latter must be numbered *B. typhosus*.

I have to thank my colleagues at the Lister Institute, particularly Dr Ledingham, Dr Henderson Smith and Dr Petrie, for much valuable help and advice during the course of these investigations. To Prof. Bainbridge, Dr Arkwright, Dr Penfold and Dr Macalister my thanks are also due, inasmuch as they helped to smooth over many difficulties both theoretical and practical.

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# A REPORT ON THE OUTBREAK OF THE PLAGUE IN COLOMBO. 1914—1916.

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(With five maps and one chart.)

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## I. INTRODUCTION.

ALTHOUGH plague broke out in India in 1896, it was not until a period of 18 years had elapsed that it reached Ceylon, notwithstanding the close proximity and the daily intercourse which takes place between the two countries. It is not surprising that this prolonged immunity, coupled with the example of the failure of the disease to thrive in such places as Madras, Singapore and other maritime towns within the tropical belt, should have created in the minds of the public in Colombo a feeling of considerable security as regards invasion by this disease, and that when it was announced that plague had at last broken out, there was the usual scepticism expressed as to the accuracy of the diagnosis. The advent of plague in Ceylon has already been announced by Castellani and Philip (1914).

In order to appreciate the unusual circumstances which attended the appearance of the disease in Colombo it is necessary to briefly describe the measures which were in force for the detection and prevention of plague prior to the arrival of the disease.

When plague first broke out in India the Government of Ceylon appointed a Committee for the purpose of adopting measures for keeping the disease out of the island. The measures adopted included the making of regulations, and the carrying out of a system of inspection and quarantine of immigrants from infected areas. Within the town of Colombo, the municipal authorities instituted a system of rat capture and destruction, whereby some 50,000 to 60,000 rodents

were secured annually. From February 1912, when the municipality first employed a bacteriologist, up till September 1913, a systematic bacteriological examination of rodents for plague was carried out by the junior author, some 2000 animals being thus examined, without, however, the finding of a single case of plague infection. From September 1913 until 7th February 1914, the bacteriological examination of rats had, for unavoidable reasons, to be suspended; but the Public Health Department Staff, being fully alive to the possibility of plague being brought over from India, were constantly on the look out for the usual signs of the presence of an epizootic. The junior author had paid special attention to this question, and had already pointed out, that the climatic conditions in Colombo were not unfavourable to plague during the months of December and January.

The first indication that anything unusual was happening, was contained in an anonymous letter, received on 24th January at the Public Health Office, in which it was stated that the residents of Sea Street were becoming alarmed at the occurrence of a number of sudden deaths, thought to be due to a new and deadly disease. The symptoms were stated to be "Fever in the morning, and death in the evening with a boil." Sea Street is a crowded insanitary quarter, lying adjacent to the harbour, which was moreover the chief centre of the grain trade between India and Ceylon.

As plague was the only new and deadly disease which was likely to appear in Colombo, and as the mention of a "boil" suggested bubos, the matter was at once treated seriously and a house to house inspection made in the locality, particular attention being paid to the search for dead rats. Nothing unusual was however found, not so much as a single dead rat, but it was ascertained that the residents, particularly the Chetties, who are the chief dealers in grain, were genuinely alarmed. An examination of the death returns and the result of interviews with medical practitioners in the locality, disclosed nothing beyond the fact that there had been some ten deaths in this locality during the previous fortnight, which however were ascribed to pneumonia, and other usual causes. It was nevertheless decided to lose no opportunity of detecting plague should it have actually broken out, and accordingly the Medical Registrar of Deaths (Dr Miss Rudd) was requested to withhold the death certificate and to at once communicate with the Public Health Department, should any further sudden death occur.

The very next day, *i.e.* on 25th January, Dr Rudd reported another sudden death in a resident of this same street, the history of which was

as follows. The patient, a well nourished and otherwise healthy young Moorish trader, who had lived in Sea Street for two months, took suddenly ill with "fever" at 10 p.m. on the night of 24th January. Next morning at 7.30 he walked to Dr Rudd's private dispensary—a distance of about half a mile. He had no symptoms at all beyond complaining of having "fever." His temperature was found to be 103° F., and he was given a mixture and walked home again. He died suddenly the same afternoon at 4 p.m. after an illness of only 18 hours. An external examination of the body disclosed nothing to account for death, and there was no trace of enlarged glands. In spite of considerable difficulties a post-mortem was held at midnight, but nothing abnormal was observed beyond a very slight congestion of the vessels of the meninges and a slight enlargement of the spleen. Samples of blood and of the various organs were taken for bacteriological examination by Dr Castellani who, in the absence of the junior author in India, had kindly consented to carry out the bacteriological investigation. Pending the receipt of the bacteriological result, steps as for plague were adopted, the house of the deceased being treated with a mixture of kerosine and cyllin.

The bacteriological examination disclosed the fact that the blood was swarming with *B. pestis*.

Within the next two weeks there was a succession of 19 sudden deaths, all but one of which were proved by bacteriological examination to be due to septicaemic plague. The exception, which was the fourteenth death in order of occurrence, developed an axillary bubo, the patient being a hospital cooly who had apparently been inoculated with the disease while engaged in a post-mortem examination of one of the previous septicaemic cases.

It was at first thought that with the decline from its initial virulence the prevailing type of infection would change from the unusual septicaemic form to the ordinary bubonic form. Reference to Tables 1 and 2 (p. 540) will show, however, that there has been practically no change in this direction in the last two years.

#### *Unusual features of the outbreak of Plague at Colombo.*

Plague is responsible for only a small mortality among the human population of Colombo, the total deaths from human plague being 383 in 1914, and 128 in 1915. The following features of this epidemic are however sufficiently remarkable and unusual to render them worthy of careful study.



(a) The percentage incidence, both among human beings and rats, is low except amongst the rats at the commencement of the epizootic, yet the virulence of the infection is very great.

(b) The clinical signs in the human subject are often very obscure.

(c) There is frequently a remarkable absence of distinctive post-mortem signs, not only in the human subject, but in both naturally and artificially infected rodents.

(d) The septicaemic type of infection prevails over the bubonic among naturally infected *Epimys rufescens*, the Colombo house rat, as it does in the human subject.

(e) The usual method of estimating the incidence of plague among rats by a study of their post-mortem appearances, which has proved so successful in Northern India, would for this epizootic fail to detect a large proportion of the most heavily infected rats.

(f) The usual signs of the presence of an epizootic among rats have been conspicuous by their absence both before and since the appearance of plague.

(g) The predominant rat flea is a different species from that occurring on rats in most plague infected localities.

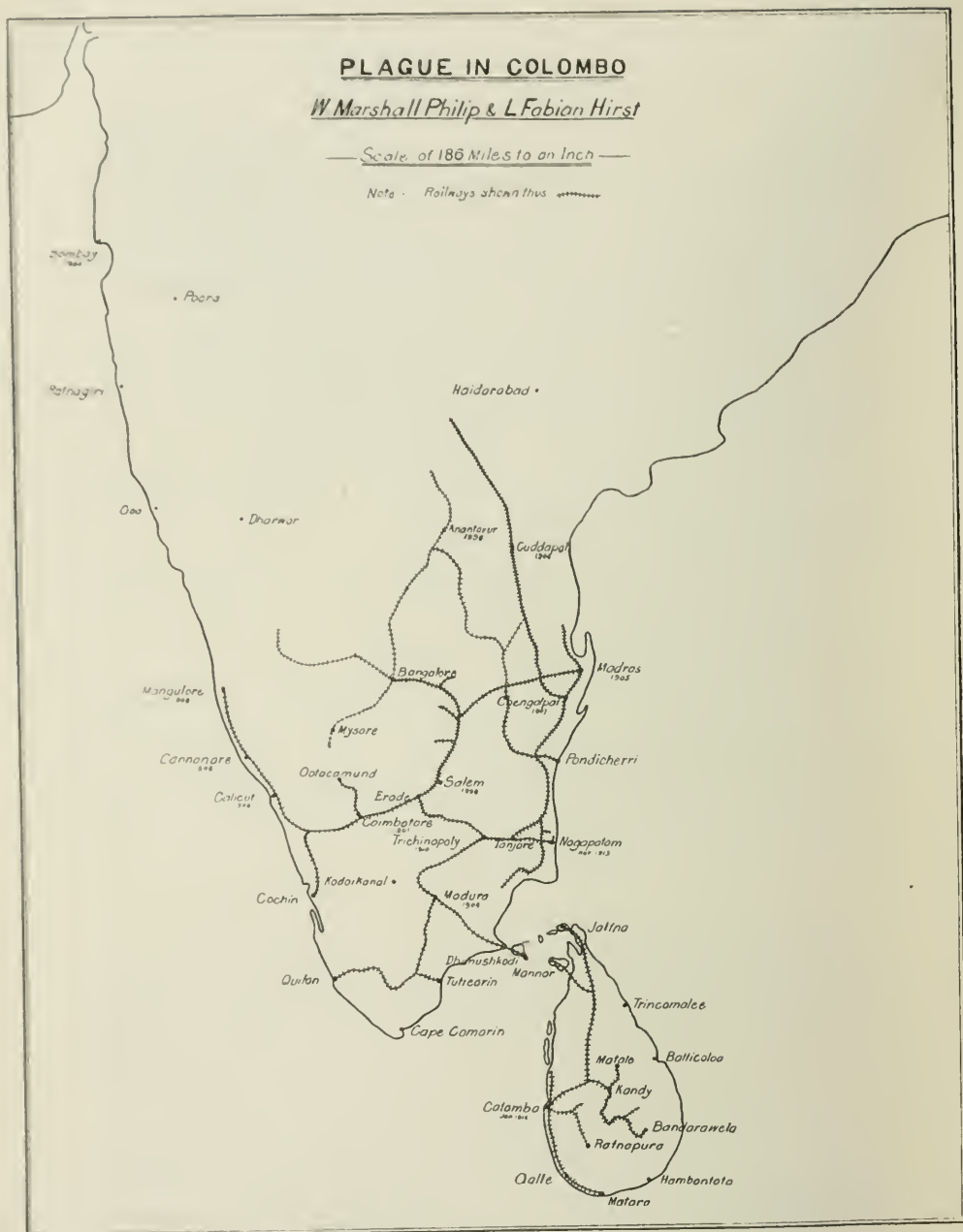
(h) There is experimental evidence that the Ceylon strain of plague is peculiar in some respects.

(i) Plague as it appeared in Colombo is a disease which might easily escape detection for long periods in localities where an efficient system of inquiry into the cause of deaths is not in operation.

(j) The incidence of the human plague has been almost entirely confined to certain insanitary districts of the city.

#### *Source of infection.*

A reference to the annexed map of South India (Map I), and Chart indicates that, as the Hon. Mr F. Bowes, C.C.S., C.M.G., Chairman of the Plague Committee, has pointed out in his report for 1914, there can be no reasonable doubt that the infection was brought from Negapatam, where a severe outbreak occurred at the end of November 1913, and reached its maximum in January 1914, in which connection he records that large shipments of rice were received from that port in late December and early January. On the assumption that the disease was conveyed by an infected rat concealed in a bag of rice, the first appearance of the disease in the grain centre at Sea Street would be explained.



Map I

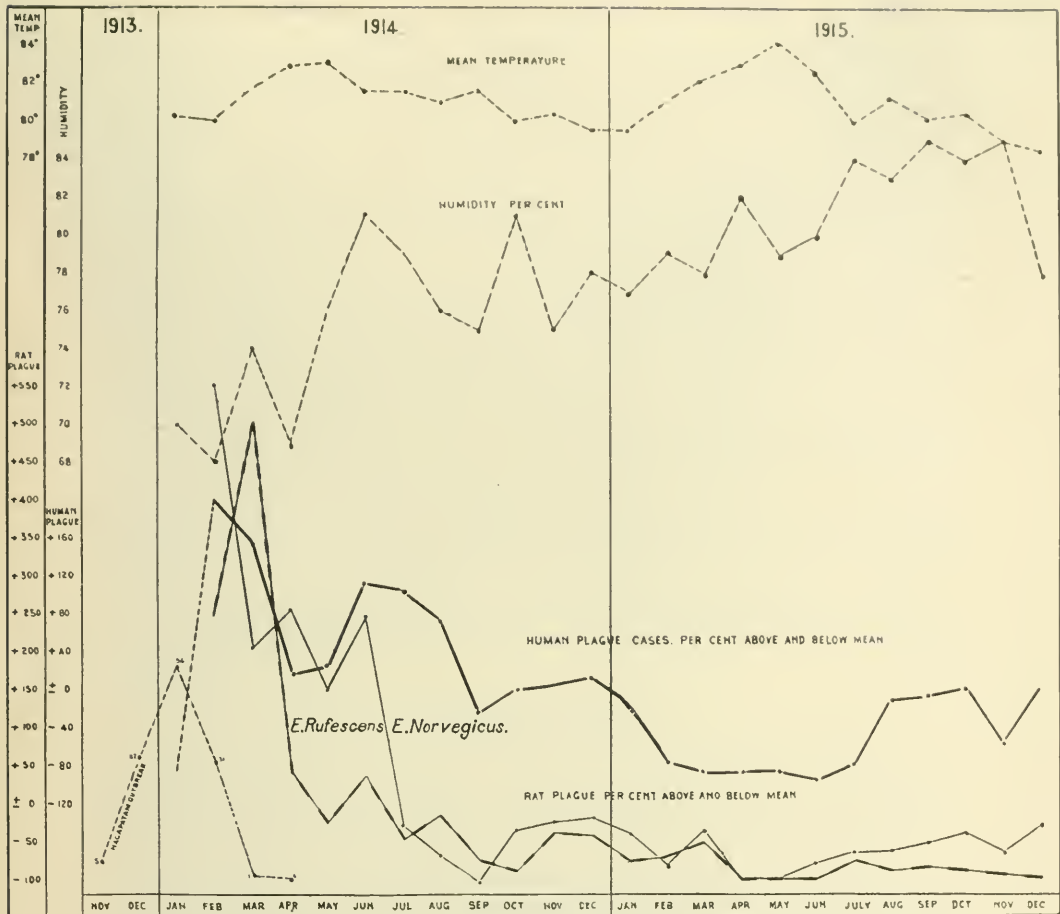


Chart showing seasonal incidence of Plague in Man and Rats.

## II. HUMAN PLAGUE IN COLOMBO.

### (a) Noteworthy features of Plague in the human subject.

*Bubonic Type.* The bubonic cases displayed no unusual features in the clinical picture or in their morbid anatomy. The case mortality is very high, and the disease seems to run an exceptionally rapid course. Bacteriologically the degree of septicaemia was unusually high.

*Septicaemic Type.* Some authorities contend that it is not correct to speak of a "septicaemic" as opposed to a "pneumonic" and "bubonic" variety of plague for the reason that septicaemia exists in every type

of plague. "Septicaemic plague" is however a convenient expression to represent a definite clinical and pathological entity quite distinct from the ordinary varieties of plague. It is associated nearly always with the early demise of the patient, probably before the usual inflammatory reactions and necrotic changes in the tissues have time to develop. It may be suggested that the high proportion of septicaemic cases recorded in Colombo is due to a large number of mild non-fatal bubonic cases having escaped detection, but the house-to-house visitations which were carried on in the infected localities were so frequent and so thorough that we feel sure that such an explanation may be rejected. On the other hand it seems not improbable that, on account of the lack of physical signs in this type of disease, rapidly fatal septicaemic cases may sometimes be overlooked in other places, thus giving a fallaciously low case mortality.

In the septicaemic plague, death is frequently very sudden, and as there are usually few indications of the real cause of death, it is the rule for the Coroner to hold an enquiry, and for the Judicial Medical Officer to perform a post-mortem on these cases.

One of us has been present at many of these autopsies, and we are in all cases furnished with notes of the post-mortem appearances.

#### *Plague septicaemia.*

In view of the comparative rarity of the purely septicaemic variety of plague it may be advantageous to give a brief description of the morbid anatomy.

As in the first case thus examined there may be a remarkable paucity of noteworthy lesions of any description.

The following are the morbid appearances most commonly noted: Marked congestion and slight oedema of the lungs. Congestion of the mucous membranes of the alimentary canal and bronchi, frequently accompanied by haemorrhagic extravasation between their coats. Marked congestion of the meninges and the cortical vessels of the brain. Petechial haemorrhages in the serous membranes of the pleural, pericardial and peritoneal cavities. Haemorrhagic extravasation into the perirenal tissue and into the pelvis and tubules of the kidney. Slight general enlargement and deep congestion of the lymphatic glands. The degree of enlargement is seldom sufficient to render it possible to easily palpate the subcutaneous lymphatic glands. The liver and spleen are usually somewhat congested, but the spleen is seldom notably



enlarged in this group of cases; *B. pestis* usually swarms in both organs.

In general the post-mortem appearances are strongly suggestive of a haemorrhagic septicaemia, but in many of the cases in which the macroscopic changes were most obscure the tissues swarmed with *B. pestis*. Marked necrotic changes are only seen in the bubonic cases.

It would be impossible to arrive at a definite diagnosis in the septicaemic cases without a bacteriological examination, though signs of haemorrhages in the tissues, the darkly congested lungs and injected mucous membranes usually give a clue to the diagnosis.

It is of interest to note the large numbers of bacilli in the alveolar cavities of the lung and in the urinary tubules. Nevertheless multiple infection among the members of a household is rare. In a few cases of septicaemic plague one lung showed a patch of consolidation but in each case pneumococci were present in large numbers in addition to the plague bacillus. They also accompanied *B. pestis* in the internal organs in smaller number. No instance of true pneumonic plague has yet been discovered in Colombo.

The bodies of persons who have died of septicaemic plague have usually been well nourished and apparently normal in outward appearance.

Material from 374 post-mortems has been examined by us and 202 positive results obtained. Of these 193 were septicaemic.

Included among the septicaemic class are a small group of cases of particular interest. In these only a few plague bacilli are visible microscopically in the tissues, and if many saprophytes are also present it may be impossible to make even a provisional diagnosis with the aid of the microscope, and it is usually necessary to resort to the animal inoculation tests. Though the organisms are so few, marked congestion of the viscera is the rule, and sometimes haemorrhages are found. The striking feature is the rapidity with which death follows the onset of symptoms, which are very indefinite, consisting merely of a slight sensation of fever and a painful feeling of oppression in the chest. The following are the registered numbers of cases which undoubtedly belong to this group and in which we have definite information of the duration of illness prior to death.

No. 17, nine hours ill; No. 122, twenty-four hours; No. 62, seven hours; No. 244, twenty-four hours; No. 590, twenty-four hours; No. 7, seventeen hours; No. 27, ten and half hours; No. 42, one hour.

For the routine examination for suspected human plague a portion of the lung, liver, and notably enlarged lymphatic glands, and the spleen are in all cases forwarded with the least possible delay from the post-mortem room to the laboratory.

In all cases smears are made from the various tissues, fixed in alcohol and stained and examined microscopically for *B. pestis*.

Our routine stain is carbol methylene blue, but if the material is fresh the prettiest preparations are obtained by staining with dilute Giemsa.

The microscopic examination enables a provisionally positive opinion to be given without delay in the great majority of the septicæmic cases.

Cultivations are also made on agar and in broth. When the suspected tissues are reasonably fresh there is seldom any difficulty in isolating the typical bacillus from these cultures. Should the microscopic examination be negative a guinea-pig is inoculated from an emulsion of the suspected tissues. The inoculation is usually subcutaneous, but if the material is much decomposed the cutaneous method is employed in order to avoid the possible pathogenic effects of some of the putrefactive organisms.

Out of twelve cases in which the body was discovered with the viscera in an advanced state of decomposition only one gave a positive result. It is possible that the thermo-precipitin test might have given a positive result in some of these cases. Recently advantage has been taken of a favourable opportunity to test the value of the thermo-precipitin method for the routine diagnosis of plague.

Three splenic punctures were performed on the dead bodies of women of the Mohammedan community. One gave a positive result. This procedure is useful when serious objections are raised to a post-mortem, but it cannot be considered reliable.

Two of the negative human cases were of special interest. In both the lungs were partially consolidated. All the tissues examined were crowded with a bipolar-staining gram-negative bacillus bearing some resemblance to that of plague but more regular in outline and less rounded at the ends. A pure culture of the organism proved very pathogenic to guinea-pigs, rats and rabbits, producing post-mortem appearances somewhat suggestive of plague. The tissues at the site of injection showed marked necrosis, the subcutaneous tissues were oedematous and congested. There was slight enlargement and congestion of the superficial lymphatic glands and as a rule some clear

fluid in the pleural and pericardial cavities. All the tissues swarmed with bacilli similar to those observed in the human tissues. On cultivation, however, the organism was at once distinguishable from *B. pestis*. It produced a dense white shining growth on agar similar to *B. lactis aerogenes* or Friedländer's bacillus. It fermented glucose, mannite, saccharose, lactose and adonite with copious production of acid and gas. It did not ferment dulcitate. The bacillus was non-motile, Vosges and Proskauer's reaction was marked, and indol negative.

The characters of this organism correspond most closely to *B. lactis aerogenes*, and it is probably allied to Friedländer's pneumonia bacillus. Similar cases have been described by Hirschbruck and Ziemann (1913).

In these instances neglect to isolate the organism and study its characters in pure culture might have led to a misleading diagnosis.

(b) *The clinical signs and symptoms in the septicæmic cases.*

The paucity of signs or symptoms of disease in cases which rapidly prove fatal is one of the most striking features of the septicæmic class of case met with in Colombo. In several instances the onset was so sudden that the patient (who has usually been a young adult) was actually pursuing his ordinary occupation without complaint on the day of his death, and only began to feel ill a few hours before the end. The following is a typical account of an average case running a course of 36 to 48 hours from the time when the patient first began to complain of feeling ill till the time of death.

The onset is always very sudden, the patient feels very ill and complains of fever and a feeling of tightness and oppression in the thorax. The patient is frequently found sitting up, if seen early in the attack, and does not at first sight appear to be very seriously ill. His face has however an anxious look. The eyes are slightly bloodshot, the breathing is hurried, the skin is hot and dry and the pulse is accelerated. Upon taking the temperature it is found to be raised, a usual figure being 103° F., but temperatures as high as 106° and even 107° have been observed. The only subjective symptoms which can as a rule be elicited are a feeling of illness and fever, and of great oppression in the chest, this latter being one of the most constant symptoms of these septicæmic cases. As a rule a question will elicit the fact that there is headache, but this is seldom volun-

tarily complained of. The outstanding complaint, and which always arouses a suspicion of plague, is the feeling of oppression in the chest. Within a few hours restlessness and delirium supervene, and the patient rapidly sinks into a state of collapse and dies.

It is remarkable how often, among the comparatively few septicæmic cases which survived long enough to reach hospital, the temperature chart shows a sharp fall to normal or subnormal about 12 hours before death, with a sharp rise just before that event took place.

As a rule there are no other symptoms than those mentioned above, but in a few cases there has been obstinate vomiting and in others diarrhoea, or both. In a small group of cases, there seems, so far as we have been able to judge by disconnected observations in the patients' homes, to have been little or no febrile reaction, the patient having rapidly reached a condition of profound toxæmia and collapse at a very early stage. Such cases are extremely puzzling, and cannot possibly be diagnosed without a bacteriological examination.

In no case included in the group of septicaemic plague has it been possible to detect by palpation any enlargement or even tenderness of the glands. This is in marked contrast to the bubonic cases where even the slightest degree of perceptible enlargement is as a rule attended with great tenderness and pain.

In no case have cutaneous hæmorrhages ever been observed in our cases.

We are indebted to Dr E. R. Loos, the Junior Assistant Medical Officer of Health, and to Dr Iyappen, the Medical Officer in charge of the Infectious Diseases Hospital for particulars in regard to the clinical features of the cases seen by them.

(c) *Incidence in relation to race, class and habits of the people.*

Race has apparently no influence *per se* upon the incidence of plague, but on the other hand "class," i.e. social position, has a very marked influence. Nearly all the cases occurred amongst the very poorest class of the population, who by reason of their poverty are compelled to live in the most insanitary parts of the town, where the conditions are most favourable to rat infestation. Although by far the largest number of cases occurred amongst Tamils, Sinhalese, and Moors, while Burghers and Malays had very few cases and



Europeans none at all, this incidence has nothing to do with race, but is merely an index of the low social condition of those affected, as proved by the fact that the wealthier members of the Tamil, Sinhalese and Moorish populations who live under more sanitary conditions were not affected at all.

Closely associated with the incidence of plague in relation to class is the question of the habits of the people. It is notorious all the world over that the poorest people are the most improvident and the most careless in matters of domestic sanitation. The well-to-do man has, it is true, his servants to collect the kitchen waste and put it in the dust bin out of sight and out of reach of rats, whereas the poor man has to do this himself, a task which he but rarely fulfils; he prefers instead to adopt the easier and more insanitary method of throwing it out on the yard. The result is that one sees more waste food-stuffs lying about in the slums than in other parts of the town, and the rat has consequently less difficulty in obtaining his meals in the poorer quarters.

Another habit amongst the poorer classes which favours the spread of plague, and one which is in a measure the direct result of poverty, is their custom of sleeping upon the floor. In the vast majority of instances it was found that the infected person slept upon the earthen floor, within easy reach of the infected rat fleas emerging from the rat holes which are most commonly situated at the junction of the floor with the wall. It not infrequently happened that the person who slept on the earth floor of the rat-riddled back kitchen became infected while the rest of the family who slept in the paved front room—possibly on a raised bed—escaped.

In this connection it is interesting that in Singapore where there has been very little plague compared with Colombo, we understand that practically all the floors even in the poorest quarters are cemented or otherwise paved, while the walls are constructed of bricks set in mortar, and it is comparatively rare to find rat holes in the houses.

(d) *Incidence in relation to sex.*

Only 113 females as against 437 males were attacked. A similar disproportion in the incidence amongst males and females is recorded in India, and it has been suggested that there it is due to greater concealment of female cases. This would not however we believe explain the extraordinary disproportion in Colombo, where the

system which is in force of registration of deaths and medical inspection of all bodies prior to the granting of a burial certificate would almost certainly have disclosed any marked tendency towards concealment. A more probable explanation is, we believe, to be found in some difference in the habits of the respective sexes, especially as regards the places where they sleep at night when rats and their fleas are abroad in search of food.

(e) *Incidence in relation to age.*

The largest number of cases occurred in persons between the ages of 10 and 25, the very young and the elderly being comparatively slightly affected. A similar incidence was observed in India, and it would appear probable that those at the extremes of life are in reality less susceptible than the full blooded young adult.

TABLE 1. *Human Plague in Colombo.*  
*Cases and deaths.*

						1914	1915
Total cases	...	...	...	...	...	412	138
Total deaths	...	...	...	...	...	381	128
Septicaemic cases	...	...	...	...	...	246	80
Septicaemic deaths	...	...	...	...	...	246	80
Bubonic cases	...	...	...	...	...	166	58
Bubonic deaths	...	...	...	...	...	135	48

TABLE 2.  
*Race distribution of cases.*

						1914	1915
Burghers	...	...	...	...	...	2	3
Sinhalese	...	...	...	...	...	108	24
Tamils	...	...	...	...	...	172	79
Moors	...	...	...	...	...	105	29
Malays	...	...	...	...	...	10	1
Others	...	...	...	...	...	15	2
All races	...	...	...	...	...	412	138
Case rate per 1000 population (all races)	...	...	...	...	...	1.72	0.56
Death rate per 1000 population (all races)	...	...	...	...	...	1.59	0.52
Percentage of septicaemic cases to total cases	...	...	...	...	...	59.7	58.0
Percentage of bubonic cases to total cases	...	...	...	...	...	40.3	42.0

N.B. In 1914 and again in 1915 one case returned as septicaemic plague "recovered," but as the diagnosis was not confirmed bacteriologically each of these has been excluded from the statistics here dealt with.

TABLE 3.  
*Age and sex distribution of cases.*

		1914			1915		
		Persons	Males	Females	Persons	Males	Females
0 years	— 5 years	13	7	6	1	1	—
5 "	— 10 "	22	11	11	5	3	2
10 "	— 15 "	72	49	23	15	14	1
15 "	— 20 "	72	58	14	22	21	1
20 "	— 25 "	63	57	6	26	26	—
25 "	— 30 "	33	26	7	10	7	3
30 "	— 35 "	51	42	9	15	13	2
35 "	— 40 "	25	19	6	15	12	3
40 "	— 50 "	24	20	4	19	16	3
50 "	— 60 "	23	16	7	10	8	2
60 "	and over	14	11	3	—	—	—
All ages ...		412	316	96	138	121	17

TABLE 4.  
*Distribution of human cases by Wards.*

			1914		1915	
Ward			Cases	Deaths	Cases	Deaths
Fort ...	...	...	1	1	0	0
Pettah ...	...	...	29	28	21	19
San Sebastian ...	...	...	48	47	15	14
St Paul's ...	...	...	156	148	69	65
Kotahena ...	...	...	12	12	11	8
New Bazaar ...	...	...	16	16	8	8
Maradana ...	...	...	112	97	2	2
Slave Island ...	...	...	26	22	6	6
Kollupitiya...	...	...	4	3	2	2
East Extension ...	...	...	3	3	3	3
Wellawatte...	...	...	0	0	0	0
Homeless vagrants			5	4	1	1
Total ...	...	...	412	381	138	128

### III. THE EPIZOOTIC.

#### (a) *Work prior to the Epizootic.*

The systematic examination of rodents in the laboratory was begun on a small scale in February 1912. Between the beginning of that month and of September 1913, 1722 rodents were examined.

In general one live rat was sent from each ward daily, together with all dead rats found within the city limits. The rats were examined for plague, trypanosomiasis and the rat leprosy of Stefanski. Collections of the various species of ecto-parasites were forwarded to the British Museum for identification.

All rats exhibiting post-mortem appearances in any way suggestive of plague were very carefully examined with the aid of the microscope, by cultivation from suspected tissues, and by animal inocula-

tion. Watch was also kept for the *B. pestis* in the many hundred slides from normal rats made for the trypanosomiasis examination. The results were entirely negative.

About 20 % of the rats examined in February and March, 1912, were infected with the *Trypanosoma lewisi*. Trypanosomiasis proved to be frequently associated with enlargement and congestion of the subcutaneous lymphatic glands, particularly the submaxillary and inguinal, and of the neighbouring tissues, and with enlargement of the spleen.

During the last quarter of 1914, only twenty rats were examined for plague owing to the extreme pressure on the resources of the laboratory as a consequence of an outbreak of cholera.

(b) *The discovery of Rat Plague in Colombo.*

The junior author resumed work at the laboratory on the 7th February 1914, and two days later he reported the discovery of rat plague in two rats from Sea Street. One of the first steps undertaken as the result of this discovery was the reorganisation of the work of rat collection and examination on a much larger scale than hitherto. The work of collection was placed under the direction of Dr A. G. Milne of the Government Sanitation Department who was seconded for the purpose as he had previous experience of plague elsewhere. On Dr Milne's departure from the Colony, this work was taken over by Mr C. W. Pate, the Municipal Veterinary Surgeon, to whom we are indebted for the statistics in regard to rat capture.

The number of rats trapped and found dead during the two years under review was as follows.

TABLE 5. *Rats trapped and found dead.*

		1914	1915
Rats trapped	...	126,394	147,198
Rats found dead	...	430	260
		126,824	147,458

TABLE 6.

*Particulars of species received at the Laboratory.*

		1914	1915
<i>Epimys rufescens</i>	...	12,833	16,505
<i>Epimys norvegicus</i>	...	4,535	6,338
<i>Mus musculus</i>	...	137	376
<i>Bandicota malabarica</i>		68	134
<i>Gerbillus indiens</i>	...	6	1
		17,579	23,354



Thirty specimens of the Insectivore, *Crocydura coerulea*, the common "musk rat" of Colombo were received in 1914, and 163 in 1915. Classed with *Epimys norvegicus* are a considerable number of *Ganomys gracilis*, possibly as much as 10 % of the total.

*Epimys rufescens* is the local representative of "*Mus rattus*" and *Epimys norvegicus* of the "*Mus norvegicus*" of many writers on plague. Types of our species have been identified for us by the Mammal Department of the British Museum (Natural History) and by the Bombay Natural History Society. In what follows we shall use the symbol E.R. to represent the former and E.N. for the latter rodent.

#### IV. GENERAL SUMMARY OF THE RESULTS OF THE RODENT EXAMINATION FOR PLAGUE INFECTION.

TABLE 7.

	<i>Epimys rufescens</i>		<i>Epimys norvegicus</i>	
	1914	1915	1914	1915
Total number rodents examined				
for plague ... ..	11,183	13,757	3,891	5,129
Total number rodents infected				
with plague ... ..	127	33	106	30
Gross percentage incidence ...	1.13 %	0.23 %	2.72 %	0.58 %
Number rodents trapped alive...	10,996	13,623	3,773	5,009
Number infected with plague...	103	27	90	24
Percentage incidence ... ..	0.93 %	0.19 %	2.38 %	0.47 %
Number rodents found dead ex-				
amined for plague ... ..	187	134	118	120
Number rodents found dead in-				
fected with plague ... ..	24	6	16	6
Percentage incidence ... ..	12.83 %	4.47 %	13.56 %	5 %

#### *Plague infection among*

	1914	1915
<i>Mus musculus</i> ...	4	0
<i>Bandicota malabarica</i>	3	2
" <i>Crocydura coerulea</i> "*	Nil	Nil

	<i>Epimys rufescens</i>		<i>Epimys norvegicus</i>	
	1914	1915	1914	1915
Septicæmic type ...	61	20	22	9
Bubonic type ... ..	66	13	84	21
Percentage septicæmic	45.08	60.6	20.75	30

\* The capture of *Crocydura coerulea* was subsequently forbidden, and all that entered the traps were liberated.

TABLE 8.

Percentage of infected trapped rodents.

Month	1914			1915		
	<i>Epiung rufescens</i>			<i>Epiung rufescens</i>		
	No. of rodents trapped	No. in fected	Per-centage	No. of rodents trapped	No. in fected	Per-centage
January	87	4	4.59	—	—	—
February	353	19	7.51	74	6	8.11
March	496	9	1.81	312	12	3.84
April	630	6	0.95	361	16	4.43
May	942	16	1.69	317	10	3.15
June	1376	9	0.65	348	15	4.31
July	1352	14	1.03	345	3	0.87
August	1624	5	0.31	428	9	2.10
September	1540	2	0.13	395	10	2.53
October	1487	11	0.74	370	3	0.81
November	1109	8	0.72	326	3	0.92
December	—	—	—	297	3	1.01
Total and Average %	10996	103	0.93	3573	90	2.52
				13623	27	0.19
				5009	24	0.47

The most remarkable feature of the Colombo epizootic is the high proportion of plague infected E.R., showing the septicaemic type of plague, viz. 45.08 % in 1914 and 60.6 % in 1915. The feature is less marked in the case of E.N., but is still very notable, viz. 20.75 % in 1914 and 30 % in 1915. The term "Bubonic Type" is used in this connection somewhat arbitrarily and includes all rats showing not only bubos but any other distinctive post-mortem sign of plague.

Another very remarkable point is the small total number of rats found dead considering the high percentage incidence. The total number for 1914 is 430, and for 1915, 320. The figures in the tables refer to examinable rats. Very large numbers of rats must nevertheless have died of the effects of both plague and rat poison, for out of 2,595,212 poison baits laid in 1914, no less than 467,814 were consumed, the figures for 1915 being 3,913,944 and 734,697 respectively. The "Punjab vermin exterminator" is used.

It is noteworthy that the spectacle of plague-infected rats wandering in the open in an obviously moribund condition, so frequently observed in India, has so far not been observed in Colombo. Rat carcasses are easily concealed in Colombo owing to the structure of the roofs of the native dwellings, and the numerous burrows communicating in many cases with extensive untrapped underground drains. When the roof is taken off plague infected dwellings mummified rat carcasses have occasionally been found among the tiles. The Java authorities seem to have a similar experience with bamboo roofs. The digging up of the burrows frequently reveals the presence of decomposed carcasses, but this method of search can comparatively seldom be adopted here, as it generally entails demolition.

The percentage incidence among living E.N. is between two and three times greater than among E.R., for both years under record. This is in accord with the general experience and is probably to be associated with the corresponding difference in the flea index. See Table No. 12 (p. 562).

On the other hand the proportion of infected dead rats is much the same for both species. The disease runs a slower and more characteristic course in E.N. and presumably this species comes out to die in the open less often when affected with plague.

We were fortunate in possessing a subordinate staff already trained to the work of rat collection and examination.

The first few hundred rats were subjected to a detailed examination

on the same lines as are employed in investigating the human cases. It soon became apparent that the ordinary macroscopic method could not be relied upon to detect plague in Colombo rats owing to the highly septicaemic form which the disease assumed and the comparative absence of distinctive post-mortem signs. It was also clear that if we continued to apply strict methods of investigation to the examination of the rats, we should not have time to examine a sufficient number to keep in touch with the spread of the epizootic.

Experienced workers in Bombay and many other plague infected localities can pick out the infected from the non-infected rats with the aid of the naked eye with a greater degree of reliability than by any other method, but a large proportion of the septicaemic rats in the Colombo epizootic appear almost normal to the naked eye and are accordingly not detected in the preliminary macroscopic examination.

In the routine method adopted here the macroscopic method is combined with a microscopic examination of the spleen and abnormal tissues, and the results are checked at intervals by inoculations of suspected material into guinea-pigs, and latterly by means of the thermo-precipitin test. It is not practicable to examine all of the very large number of trapped rats by such a method.

The following are examined daily:

Group 1. All dead rats found within the city limits.

Group 2. All rats found alive in the vicinity of recently infected premises.

Group 3. An equal proportion of the rats from all over the town trapped by each overseer of the Rat Collection Department. These are drowned at the Depot.

Numbered brass tickets are pinned to the bodies of the rats. A list is forwarded with the rats from the Depot showing the locality of collection for each number.

The method of examination differs somewhat and the results are interpreted in a slightly different manner according to whether the rat's carcase is fresh or decomposed.

*In fresh carcasses* reliance is chiefly placed on the microscopic examination, frequently controlled by the isolation of the specific organisms in cultures from the organs or from the tissues of guinea-pigs inoculated with organ emulsions, and latterly in the case of the more septicaemic specimens, by the thermo-precipitin test. The



carcasses of Groups 2 and 3, *i.e.* trapped rats, are nearly always fresh when received in the Laboratory. One of us is personally responsible for all but a few of the microscopic examinations.

*In decomposed carcasses* the microscopic method by itself is of little value since plague organisms rapidly disappear from the tissues at ordinary Colombo temperature and are replaced by strongly staining saprophytes which overgrow the cultures. In such cases one is obliged to supplement the microscopic method by cutaneous inoculation of guinea-pigs, and by the precipitin test. It is however impossible to obtain reliable results in the case of decomposed septicæmic rats since not only is the microscopic method of no value, but cutaneous inoculation often fails with decomposed material known to be plague infected.

In all rats not in an advanced state of decomposition the usual dissection is made by native laboratory attendants under the supervision of a trained assistant. The post-mortem appearances are carefully scheduled on forms designed to facilitate the analysis of the results. The species, sex, size, pregnancy and number of foetuses are also noted. Smears are made on glass slides from the spleen of every rat not in an advanced state of decomposition, also from such lesions as bubos, granular liver and pleural effusion.

The high degree of septicaemia usually found in plague infected Colombo rats is exceptionally favourable to the use of the microscopic method. In the great majority of infected rats, typical bacilli swarm in every field of the microscope. As in the corresponding human cases they were often particularly numerous when the macroscopic signs were inconspicuous. In the bubonic type the organisms are often less numerous and sometimes quite scarce, but here the macroscopic appearance helps in the diagnosis.

It is unsafe to give an opinion on the basis of the macroscopic examination alone when only a few bipolar organisms are noted. Bipolar staining bacilli of intestinal origin not uncommonly appear in splenic smears in small numbers quite a short time after the death of the rat. Other observers have called attention to their presence in the accessory genital glands and in non-plague pleural effusions (Macalister and Brooks, 1914). Experience however shows that when numerous bacilli of typical morphology are found, and especially if involution forms are observed, there is a very strong presumption of plague. The interpretation of the appearances observed in moderately decomposed tissues is much facilitated by a preliminary

study of the corresponding tissues of an artificially infected animal at different periods after its death.

The presence of plague infection is most likely to be overlooked when the animal dies at an early stage from the effects of plague toxins before the organisms have greatly multiplied in the tissues.

*Principal post-mortem signs observed among 127 plague-infected E.R. and 109 E.N. during 1914.*

	E.R.	E.N.
Marked subcutaneous congestion ... ..	26	62
Marked enlargement and congestion of lymphatic glands	25	48
Necrosis or suppuration of lymphatic glands ... ..	7	5
Effusion of clear fluid into pleural pericardial cavities ...	26	26
Granular liver ... ..	6	25

The most reliable single sign of plague among our rats was granular necrosis of the liver. We have not met with the necrotic liver due to infection with Gaertner's bacillus. The most generally useful sign is clear pleural effusion but in several rats with this abnormality we did not succeed in proving the presence of *B. pestis*.

Only twelve rats during 1914 showed really typical post-mortem appearances. These include rats showing at least three characteristic signs such as granular liver, pleural and pericardial effusion, bubo, subcutaneous congestion or haemorrhages.

It will be noted that the distinctive post-mortem appearances are much more common in the E.N. than in E.R.

Non-plague abscesses were noted in the lungs and in connection with the accessory genital glands in 46 rats. Many were of large size, the contents being either caseous or of a glairy consistency. The 21 non-plague abscesses of the lymphatic glands contained large numbers of a slender pointed Gram negative bacillus.

There were six cases of rat leprosy among the E.N. The non-plague morbidity of this species is much higher than that of E.R.

During February, March and April, 1914, congestion of the superficial lymphatic glands and of the subcutaneous tissues was very common in both species quite apart from plague infection. Many of these enlarged glands contain trypanosomes, and it is not unreasonable to suppose, since both infections may be conveyed by the rat flea, that the incidence of both may be influenced by similar conditions.

## V. RELATION BETWEEN HUMAN AND RAT PLAGUE.

As already pointed out the incidence of human plague has been almost confined to certain insanitary, highly rat-infested quarters of the city. These areas are fairly well defined, and it is a matter of some interest to compare the incidence of rat plague at different periods within and without these areas, during the year 1914. For this purpose three such areas were marked out on the map. (See Map III.)

Number one comprised the original focus of infection in the Pettah Ward and the adjoining portions of San Sebastian and St Paul's Wards with a continuous strip of Maradana Ward to the East of Colombo Lake. Throughout the year this remained the area principally involved.

A second area in Slave Island Ward was considered to be infected for the months of February, March and April, after which it became almost free from human plague.

A third area in Demetagoda became a focus of human plague from June to the end of the year.

The accompanying table No. 9 shows the percentage incidence of plague infection among rodents in areas infected and non-infected with human plague.

The epizootic was established in Maradana Ward and a small area of St Paul's Ward nearly three weeks before human cases occurred.

TABLE 9.

*Percentage incidence of plague infection among rodents in areas infected and non-infected with human plague.*

<i>Epimys rufescens.</i>						
	Infected area			Non-infected area		
	No. of rodents	No. infected	Percentage	No. of rodents	No. infected	Percentage
1st Quarter	192	13	6.76	248	10	4.03
2nd "	784	16	2.04	1284	15	1.16
3rd "	1960	15	0.76	2392	13	0.54
4th "	1655	9	0.54	2481	12	0.48
<i>Epimys norvegicus.</i>						
1st Quarter	231	17	7.35	155	1	0.64
2nd "	598	29	4.85	428	12	2.80
3rd "	731	15	2.05	437	7	1.60
4th "	490	6	1.22	503	3	0.59

The discovery of rat plague preceded that of human plague in fifteen streets of the town, thus enabling a warning note to be sounded and precautions were taken in advance.

In June a small focus of rat plague was discovered in the south end of the town at Wellawatte but was not followed by any indigenous cases of human plague.

It is particularly noteworthy that the rat plague when it first appeared in new areas was almost always of the septicaemic type. It is clear therefore that for practical purposes it is important to adopt a technique which will detect this class of infection and that undue reliance should not be placed upon the macroscopic method as a guide to the spread of this type of epizootic.

#### VI. RAT INFESTATION AND PLAGUE INCIDENCE IN RELATION TO LOCALITY.

A very striking relation has been observed in Colombo between the incidence of plague amongst the people, and the conditions under which they are living, particularly as regards the character of their dwellings and the situation of these in relation to grain centres and thoroughfares.

It is not merely that in its spread the disease has selected with remarkable precision and regularity the most insanitary spots in the town, but it has during the two years of its stay, remained, in so far as the people are concerned, strictly confined to such areas, although on the other hand infection amongst the rats has been found to occur in practically every quarter of the town.

The explanation of this without doubt lies in the greater degree of rat infestation which prevails, and the closer domestic relation which is tolerated between rat and man in the poorer as compared with the better class quarters of the town.

The greater degree of rat infestation in plague infected areas, and the probable reasons for such infestation are indicated in the following description of various areas in the town.

We shall consider these areas in order, inversely to the degree of rat infestation:

(a) *Business quarter, Fort (A on Map II).* 9.3 rats per 100 traps.

The buildings in this quarter are for the most part used for business purposes, and (exclusive of hotels) only to a very small extent for



dwelling purposes. They are substantially constructed, many having an upper floor. The ground floors are invariably cemented, as are also many of the back yards which are, however, small. The lighting and ventilation of such as are used for dwelling purposes is good. General sanitary condition good. No case of human plague, and very few cases of rat plague have occurred here.

(b) *Residential quarter, Cinnamon Gardens (B on Map II). 10.5 rats per 100 traps.*

The houses are for the most part single-storey detached buildings, substantially constructed of brick or cabook, plastered and linewashed, floors are cemented, roofs covered with half-round country or flat Mangalore tiles and ceiled; light and ventilation ample, and general sanitary condition good. No case of human plague, and only a few sporadic cases of rat plague have been detected in this part of the town.

(c) *Native quarter, fairly densely populated, Slave Island (C on Map II). 16.6 rats per 100 traps.*

The houses in this quarter are almost entirely small single-storey dwellings, the main building being in most cases constructed of roughly cut cabook bricks set in clay or coarse sand mortar; the foundations are similarly built and are shallow, the walls are plastered with chunam, the roofs are low and covered with half-round country tiles; there is as a rule no ceiling. The kitchens are frequently merely one end of the back verandah enclosed by wattle and daub walls. The floors are for the most part of beaten earth. The lighting and ventilation are defective, owing partly to the irregular disposition and overcrowding of the buildings, and partly to the absence of windows and the existence of interior rooms. General sanitary condition is bad. The walls of such houses are generally riddled with rat runs. A small but distinct outbreak of both rat and human plague occurred in one part of this area in 1914, but only four human and no rat cases were detected in 1915.

(d) *Native quarter densely populated, San Sebastian (D on Map II). 18.7 rats per 100 traps.*

The houses in this quarter, which occupies the side of a slope, are constructed much as in (c), but are more closely crowded together in continuous rows, and there are many long ranges of back to back tenements which are densely populated. The people are very insanitary in their habits, and the general sanitary condition of the quarter is

bad. Comparatively little rat plague has been found in this quarter, but it was the scene of a sharp outbreak of human plague in 1914. This area is in comparatively close proximity to the grain-centre area described next.

(c) *Grain Centres, Pettah and St Paul's (E on Map II).* 21.1 rats per 100 traps.

This area is the chief centre of the grain trade, and is fairly accurately indicated by the densely crowded spots at *E* on the attached map. It lies adjacent to the harbour and contains, besides the grain stores, large numbers of dwellings between which, in fact, the grain stores are sandwiched. The grain stores are for the most part nothing more nor less than dwelling houses which have been converted into stores, additional accommodation being obtained by the simple expedient of roofing over the small back yard. The buildings present a combination of the fairly well built, cement-paved house, and the badly built unpaved tenement. In practically all cases however the walls are quite pervious to rats as is shown by their being riddled with rat runs. An important feature of this area is the existence of an old underground system of untrapped rain-water drains which discharge for the most part into the harbour. It was thought early in the epidemic that the absence of dead rats might be explained by the finding of large numbers of their bodies in these drains, but an inspection proved this not to be the case. These drains however undoubtedly form great highways for the rats, and several of them have been proved to have direct communication with the interiors of the dwellings by means of rat runs.

This area was the first part of the town to be attacked by plague, and it has continued ever since to be the principal focus of the disease as regards both rat and human plague.

(f) *Semi-rural native quarter, Modera (F on Map II).* 23.3 rats per 100 traps.

Although this area shows one of the highest degrees of rat infestation, very little plague, either rat or human, occurred during the two years under reference. The houses are much the same as those described under area (c), but there is a larger proportion of the bamboo-framed wattle and daub building here, and in a number of cases either the whole or part of the house is roofed with thatch instead of tiles. The houses are more scattered than in other areas. There are no underground drains in this area. Rat holes are not as a rule so numerous as in the

other areas described which is probably attributable to the relatively small number of *E. norvegicus* here as shown by the following statement.

		Area (c)	Area (f)
Total rats examined	...	3335	1033
No. of <i>E. rufescens</i>	...	2014	864
No. of <i>E. norvegicus</i>	...	1321	169
Percent. of <i>E. rufescens</i>	...	60	83.6
Percent. of <i>E. norvegicus</i>	...	40	16.4

A distinctive feature of this area is its isolation. It forms a salient at the extreme north end of the town, and is bounded on three sides by the river and the sea, there being no through road to the country beyond. The result is that there is little traffic here compared with the other areas described. It is of interest to record that plague has appeared in this area subsequently to the period under consideration, and at the time of writing shows signs of a fairly widespread and active infection. Presumably this late appearance of the infection is due to the isolated position of the area.

(g) *Native quarter with bazaar, Maradana (G on Map 11).* 27.6 rats per 100 traps.

This area which shows the highest degree of rat infestation is an extremely busy centre. In addition to being densely populated, it is a great centre for native retail grain and food shops. It is close to the principal railway station, and one of the principal roads of the town passes through it, there being an important road junction here. This area was early attacked by plague and was the scene of a very sharp outbreak of human plague in 1914. No case of either rat or human plague occurred in 1915.

The use of the number of rats caught per 100 traps as our index to rat infestation is open to the objection that rats will not enter the traps in numbers proportionate to the actual degree of rat infestation, if there be a great amount of tempting food easily accessible to them outside the traps; and on the other hand will enter the traps in disproportionately large numbers if their usual food supply is suddenly cut off by exceptionally vigorous scavenging operations.

During the period to which the figures refer, July, August, and September, 1915, the trapping and scavenging operations were being carried out on the same uniform system. The baits used in the cages are very tempting to rats and the cages are laid in similar situations in all the areas.

## VII. CHARACTERS OF OUR STRAINS OF CEYLON PLAGUE.

All our strains are morphologically typical and show marked bipolar staining. On agar, growth appears in the form of discrete translucent streptococci-like colonies. Later the colonies usually coalesce to form a whitish somewhat opaque growth with raised colonies on the surface somewhat suggestive of a contamination. All our strains form typical stalactites in broth and grow in the form of long chains. No indol reaction to Bohme's reagent is given with any of the strains.

Many of our Colombo strains are remarkably parasitic, only growing scantily on agar or blood serum and requiring to be subcultured at frequent intervals in broth to keep them alive.

The fermentation reactions of these strains of human plague Nos. 2860, 2191 and 3870, and those of the rat plague Nos. 45, 47 and 32,188, have been carefully compared with one another and with a strain of *B. pestis* from the Bacteriological Laboratory, Bombay.

Peptone azolitmin water was used containing  $\frac{1}{2}$  per cent. of the following substances: lactose, saccharose, dulcite, adonite, inuline, salicine, dextrine, glycerine, glucose, galactose, arabinose, fructose, sorbite, isodulcite and mannite.

On the 14th day acid was formed in arabinose, glucose, fructose, galactose, maltose, mannite, salicine, dextrine and a trace of acid in adonite.

Lactose, saccharose, dulcite, inuline, raffinose, isodulcite, sorbite, and glycerine, gave no reaction.

There was no appreciable difference between the Ceylon strains. The Bombay strain fermented salicine more vigorously and adonite hardly at all.

Our strains proved extremely virulent to Colombo rats. After subcutaneous injection of an emulsion in salt solution containing only 100 organisms per c.c. of strain 3870, two *Epimys rufescens* died within 48 hours of septicaemic plague. The cutaneous inoculation of a few loopfuls of the recently isolated broth culture into the shaved skin at the root of the tail killed four out of six of this species rapidly, in one instance in less than 36 hours. Our laboratory culture of plague is also very virulent to Colombo rodents but in a less degree. In this respect Colombo seems to come into line with the City of Madras and other places in India where low endemicity of plague is associated with great susceptibility of the rat population.



A few of the experimentally infected rats which died very rapidly showed a condition analogous to the toxæmic group of human plague to which reference has been made. The organs contained only a few *B. pestis* and the macroscopic appearances were a little abnormal. The vitality of the animals was probably low with the result that they died at an early stage of the bacterial invasion of the tissues from the effects of plague toxin. The corresponding condition has been observed in specimens of naturally infected *Epimys rufescens*.

Rats inoculated from a spleen emulsion of human and rat plague almost invariably die of septicaemia within two days. The post-mortem appearances are precisely similar to those found in the naturally infected septicaemic rats.

Guinea-pigs inoculated with fresh tissue emulsion of septicaemic plague often die within 48 hours and show a corresponding condition post-mortem to septicaemic plague in rats and human beings.

Guinea-pigs inoculated from cultivations however seldom die before the third day. Microscopically the viscera show the changes typical of plague.

The virulence of No. 3870 when recently isolated was compared with the Bombay strain on guinea-pigs, using eight pigs of approximately equal weight and an emulsion of *B. pestis* containing 10, 100, 1000, 10,000 100,000, 1,000,000 of strain 3870 and 1,000,000, and 10,000,000 of the Bombay strain. The cultures were 48 hours old. The suspensions in salt solution, after shaking for 15 minutes, were standardised with a Helber Glynn Haemocytometer. The injections were subcutaneous, care being taken to deliver a uniform quantity into the tissues. The pig receiving the smallest dose survived. That receiving 1000 organisms died of septicaemic plague in 48 hours. All the remainder died between the third and fifth day with typical post-mortem signs of plague such as granular liver and effusion into the pleural cavities.

Haemorrhage into the tissues is common in guinea-pigs inoculated with our strains if they survive more than three days. In experimentally infected rats it seems as uncommon as in the natural infected animals.

A comparatively large dose of *B. pestis* is required to reproduce the septicaemic type of infection in *Epimys norvegicus*. This rodent seems to be intermediary in this respect between *E. rufescens* and the guinea-pig.

Sydney Rowland (1915), experimenting with a culture of Ceylon plague isolated by Castellani, found it to be of low virulence for his rats, only killing 50 % of those inoculated. Rowland brings forward

evidence showing that the antigen of the Ceylon strain of plague differs in immunising power from his standard laboratory strain.

It follows that prophylactic vaccines intended for use in Colombo should be prepared from the indigenous strain. It seems probable however that vaccines would not give much protection against such a virulent strain as that responsible for the local epidemic.

Sydney Rowland (1915*a*) failed to immunise rats against a hyper-virulent body strain of rat plague with the most effective vaccine he could prepare from this strain.

#### VIII. THE THERMO-PRECIPTIN REACTION.

Ascoli's thermo-precipitin test has been employed on a small scale. Our supply of suitable serum is as yet too limited to apply the test as part of our routine examination for plague.

The serum used was Yersin's antipest serum prepared at the Berne Institute.

It was often necessary to filter this serum through a porcelain candle before it became clear enough to be used.

The method is particularly useful as a rapid confirmatory test for septicaemic plague. A well-defined ring is usually visible at the junction of the fluids in about an hour with the technique recommended by C. E. Warner (1914). In our experience the results are distinctly better with fresh tissues.

In four out of seven extracts from moderately decomposed human plague spleens, and in two out of three rat spleens in a similar condition, a good reaction was obtained within two hours. In the case of the remaining three human spleens a good ring formed overnight. The other rat gave a dubious reaction.

A positive result will often be obtained by this method when it is practically impossible to isolate *B. pestis* directly from among the putrefactive organisms in decomposed plague infected tissues.

On 10th January 1916, we succeeded in obtaining a positive result with the aid of the test from five mummified rats found in the roof of No. 69<sup>11</sup> Dam Street. A human death had taken place from bubonic plague on the 6th instant at 69<sup>8</sup> Dam Street, a few yards distant in the same block of buildings.

The following are the details of this test:

An emulsion was made from the scrapings of the skin and bone of the five rats in the proportion of one gramme of scraping to four of salt solution. The emulsion was heated in a water-bath to 100° C. for five minutes, filtered first through ordinary filter paper and then through a Pasteur-Chamberland filter. A clear but discoloured filtrate was obtained. As controls similar extracts from the spleens of a plague infected rat caught in the same street and of a microscopically negative rat, after being heated in the same manner, were also passed through a sterilised filter candle. A control with rabbit serum was also prepared. Yersin plague serum from the same bulb was added to each tube.

The control positive tube showed a distinct ring within fifteen minutes. The mummified rat tube reacted distinctly in about one hour. The control negative tubes showed no reaction several hours later, but next morning a faint turbidity was visible at the junction of the fluids in the negative rat tube. By that time the two former tubes had precipitated.

The reaction seems to be sufficiently specific for practical purposes.

#### IX. PARASITOLOGY.

The following species of ecto-parasites have so far been identified from Colombo rats.

Siphonaptera	{ <i>Xenopsylla astia</i> Rothschild ,, <i>cheopis</i> Rothschild
Gamasidae	{ <i>Dermanyssus muris</i> Hirst <i>Laelaps echidninus</i> Berlese ,, <i>nuttalli</i> Hirst
Louse	<i>Haematopinus spinulosus</i>

*Dermanyssus* (*Liponyssus*) *muris* is a blood-sucking mite, *Dermanyssus gallinae* has repeatedly been found parasitic on man in Europe and America. The Dermanyssidae are generally parasitic upon birds.

The mites of the genus *Laelaps* are not parasitic upon man, but might possibly convey infection from rat to rat. Species of both *Dermanyssus* and *Laelaps* have now been found parasitic upon rats from all parts of the world.

The fleas were identified by the Hon. N. C. Rothschild, and the mites by Stanley Hirst from collections forwarded at intervals since 1912; the smallest species of mites are not included.

All the fleas in the collections forwarded to the Hon. N. C. Rothschild in 1912 were identified as *Xenopsylla astia*. In March 1914 the junior author observed some fleas which appeared to belong to a different species, probably *X. cheopis*. He included these fleas in a collection of 200 which was forwarded to Rothschild. Some of the male fleas of both species were cleared and mounted on glass slides and their characters carefully noted for future reference. One of the fleas sent had been caught on a man in the Colombo Museum, and was also cleared and noted; the remainder were from E.R. and E.N., captured in the principal area of plague infection.

Rothschild reported as follows: 28th April, 1914.

183	<i>Xenopsylla astia</i>	
16	„	<i>cheopis</i>
1	<i>Echidnophaga</i>	<i>gallinacea</i>
<hr/>		
200		

On the glass slides two *X. cheopis* and four *X. astia*.

In May 1914, one of us estimated five out of 200 male fleas to be *X. cheopis*. We have not found this species at any time subsequently.

*X. astia* was originally described by Rothschild (1911) from specimens captured in Rangoon. We obtained a collection of rat fleas from Madras City. All were identified by Rothschild as *X. astia*. One of us (Hirst, 1913) has drawn attention to the epidemiological significance of the already ascertained facts with regard to the geographical distribution of *X. astia*.

We learn from Rothschild that about 5 % of the rat fleas in Bombay are *X. brasiliensis*, and that he proposes to publish a paper explaining the differences between these species to enable students to identify them with ease.

A series of parasitological experiments with *X. astia* are in progress, but our observations have not as yet been sufficiently continuous or complete to warrant publication in detail.

Observations made in 1913 before the outbreak of plague showed that *X. astia* bites man with great reluctance at temperatures over 80° F. We have experienced the greatest difficulty in transmitting plague from rat to rat under experimental conditions through the agency of *X. astia*.



Experiments in flea breeding cages of the usual pattern are attended with great difficulty in the moist Colombo atmosphere, on account of the rapidity with which the larval food is overgrown with moulds. We have, however, devised a method by which this difficulty is overcome.

Several attempts have been made to estimate the flea infestation of infected dwellings by using guinea-pigs as traps for fleas, after the method used by Liston with such success in Bombay. Our experience was quite different. It was exceptional to find any fleas at all on the animal. The highest count obtained was seven fleas on one pig. All the exposed animals survived.

In this connection it is noteworthy that here fleas comparatively seldom attack disinfecting coolies and others entering plague infected houses after the removal of the patient. This seems to be in marked contrast to the general experience in Northern India.

Everything appears to point to the conclusion that the conditions prevailing in Colombo are exceptionally unfavourable to the transmission of plague infection, since in spite of the virulence of the infecting organisms and the susceptibility of the human and rat population, the incidence of the disease is low for both except at the very commencement of the epidemic.

It is not yet possible to decide whether this is mainly due to the effect of climatic condition on the vitality of the flea, to the relative inefficiency of *X. astia* as a porter of plague, or to any other cause.

In view of the exceptionally high degree of septicaemia in many of our cases of human plague, the question of the possibility of the transmission of the infection from man to man through the agency of human ecto-parasites such as *Pulex hominis*, *Cimex rotundatus* or *Pediculus corporis* becomes of practical importance and especially in view of the work of Nicolle and his collaborators. We are not yet, however, in a position to record any fresh data bearing on this matter.

Batches of about twenty rats are brought to the laboratory three times a week alive in canvas covered cases for the purpose of estimating the flea index. After transferring rats from any cage containing more than one species, so that all the cages contain one species of rats, the whole cage in its bag is placed in a chloroform or cyanide box and the fleas counted after combing out the rats and shaking out the bag.

## X. SEASONAL INCIDENCE.

The monthly mean temperature varies comparatively little in Colombo throughout the year. The nights are fairly cool as a rule in December and January when the mean monthly minimum atmospheric temperature may fall to 72° F.

Rise of temperature above 81° F. seems to have a decided effect on the incidence of plague.

The humidity ranged higher during 1915 than 1914. There seems to be more distinct relation between the curve of humidity and the flea index than between that of flea index and temperature.

Our curves show a very close correspondence between the periodicity of rat and human plague.

The sharp rise in the flea index (p. 562) in June 1915 was followed by a rise in the curve of plague incidence among E.N.

(See Chart on p. 533.)

TABLE 10. *Plague, 1914 and 1915.*

*Human cases reported during each month.*

			1914	1915
January	...	...	4	19
February	...	...	67	6
March	...	...	58	3
April	...	...	27	3
May	...	...	29	3
June	...	...	49	1
July	...	...	47	5
August	...	...	40	20
September	...	...	18	21
October	...	...	23	23
November	...	...	24	10
December	...	...	26	24
Totals	...	...	412	138
Mean	...	...	34	11.5

TABLE 11 (a).

*Monthly temperature and humidity at Colombo during 1914.*

Month	Temperature			Humidity (mean)
	Maximum in shade	Minimum in shade	Mean of maximum and minimum	
	°	°	°	%
January ...	88.2	72.3	80.3	74
February ...	88.2	71.4	79.8	75
March ...	89.3	74.0	81.7	78
April ...	90.4	74.9	82.7	78
May ...	88.3	77.7	83.0	79
June ...	86.3	76.9	81.6	82
July ...	86.0	77.0	81.5	80
August ...	85.9	76.1	81.0	79
September ...	87.4	75.7	81.6	71
October ...	86.2	73.8	80.0	78
November ...	87.3	73.4	80.4	72
December ...	86.0	73.1	79.6	75
Mean for year	87.5	74.7	81.1	77

TABLE 11 (b).

*Monthly temperature and humidity at Colombo during 1915.*

Month	Temperature			Humidity (mean)
	Maximum in shade	Minimum in shade	Mean of maximum and minimum	
	°	°	°	%
January ...	86.8	72.3	79.6	77
February ...	89.8	72.3	81.0	79
March ...	89.9	74.5	82.2	78
April ...	90.7	75.5	83.1	82
May ...	89.0	79.3	84.2	79
June ...	87.7	77.6	82.6	80
July ...	84.6	75.5	80.0	84
August ...	85.2	77.2	81.2	83
September ...	85.0	75.4	80.2	85
October ...	85.5	75.4	80.4	84
November ...	84.0	73.8	78.9	85
December ...	85.8	71.5	78.6	78
Mean for year	87.0	75.0	81.0	81





## XI. PREVENTIVE MEASURES.

The routine preventive measures adopted consist of removal of patients to hospital, segregation of contacts, house-to-house inspections, keeping all fever cases under observation, disinfection of the building including the use of a pulicide (crude petroleum or a mixture of kerosine and cyllin), removal of the roof to let the sun in, sulphur fumigation of rat runs by means of Clayton machines, blocking up of rat holes with broken glass, bricks, etc., and cement mortar, cleansing and limewashing of the premises generally including search for rat nests and dead rats, evacuation of insanitary tenements in infected areas pending the carrying out of structural improvements, closure of wholesale rice stores in infected areas, steam disinfection of second-hand clothes prior to despatch upcountry, capture and poisoning of rats, bacteriological examination of rats, estimation of flea index.

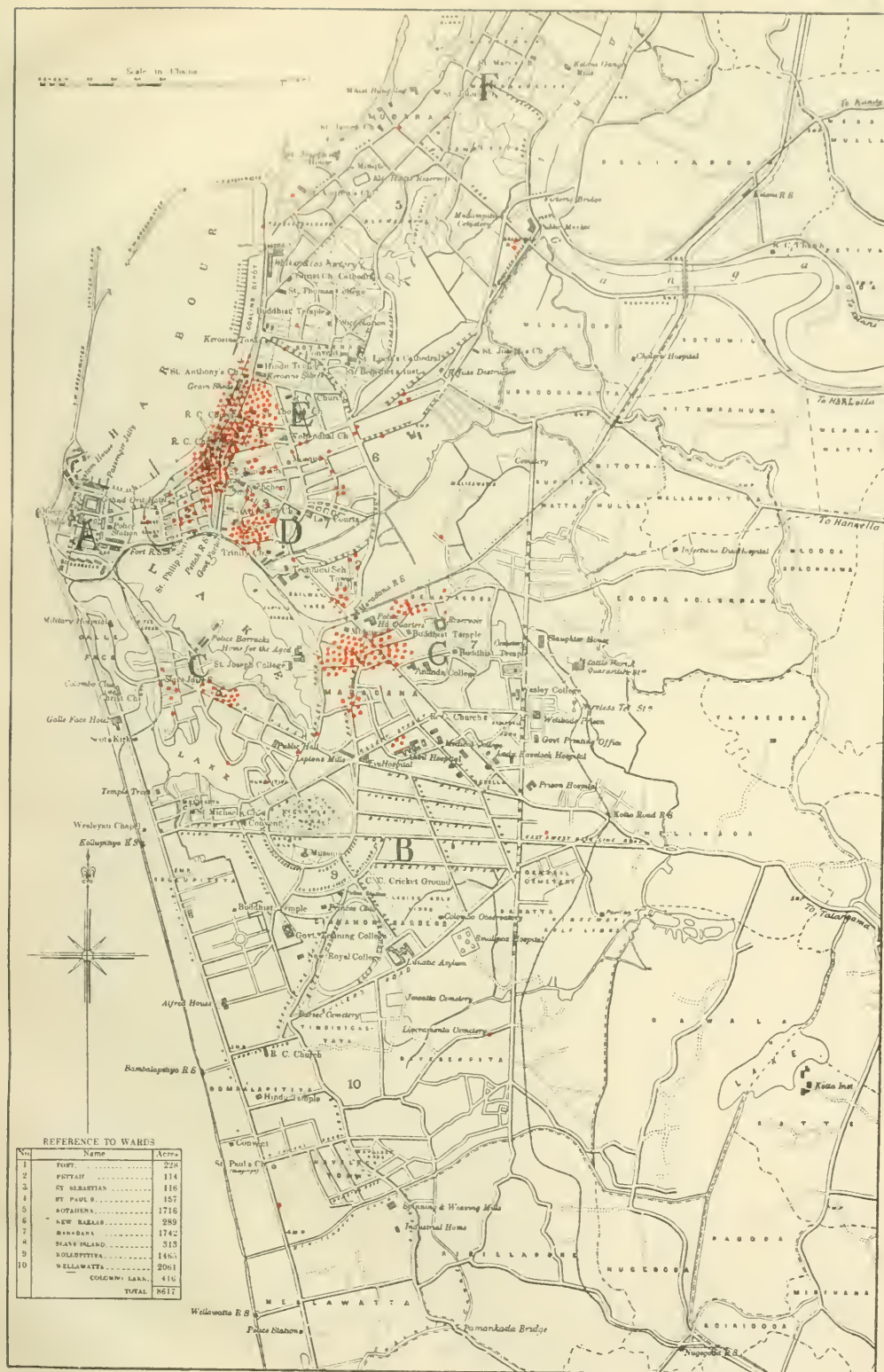
The danger associated with the rat infested privately owned wholesale rice stores has been greatly mitigated by the action of Government in erecting up-to-date rat proof warehouses for the storage of rice, and prohibiting the transport of rice upcountry from the insanitary rice stores in the town.

Fairly early in the course of the outbreak a supply of anti-plague vaccine was obtained from Bombay, and its use was widely advocated. Almost immediately, however, the wildest stories as to the danger of its use began to circulate, set afoot, it is believed, by persons operating for ulterior motives, with the result that a panic ensued amongst the cooly classes who began to bolt to India in such large numbers that a labour famine was threatened. Consequently all attempts to induce these people to be inoculated had to be abandoned.

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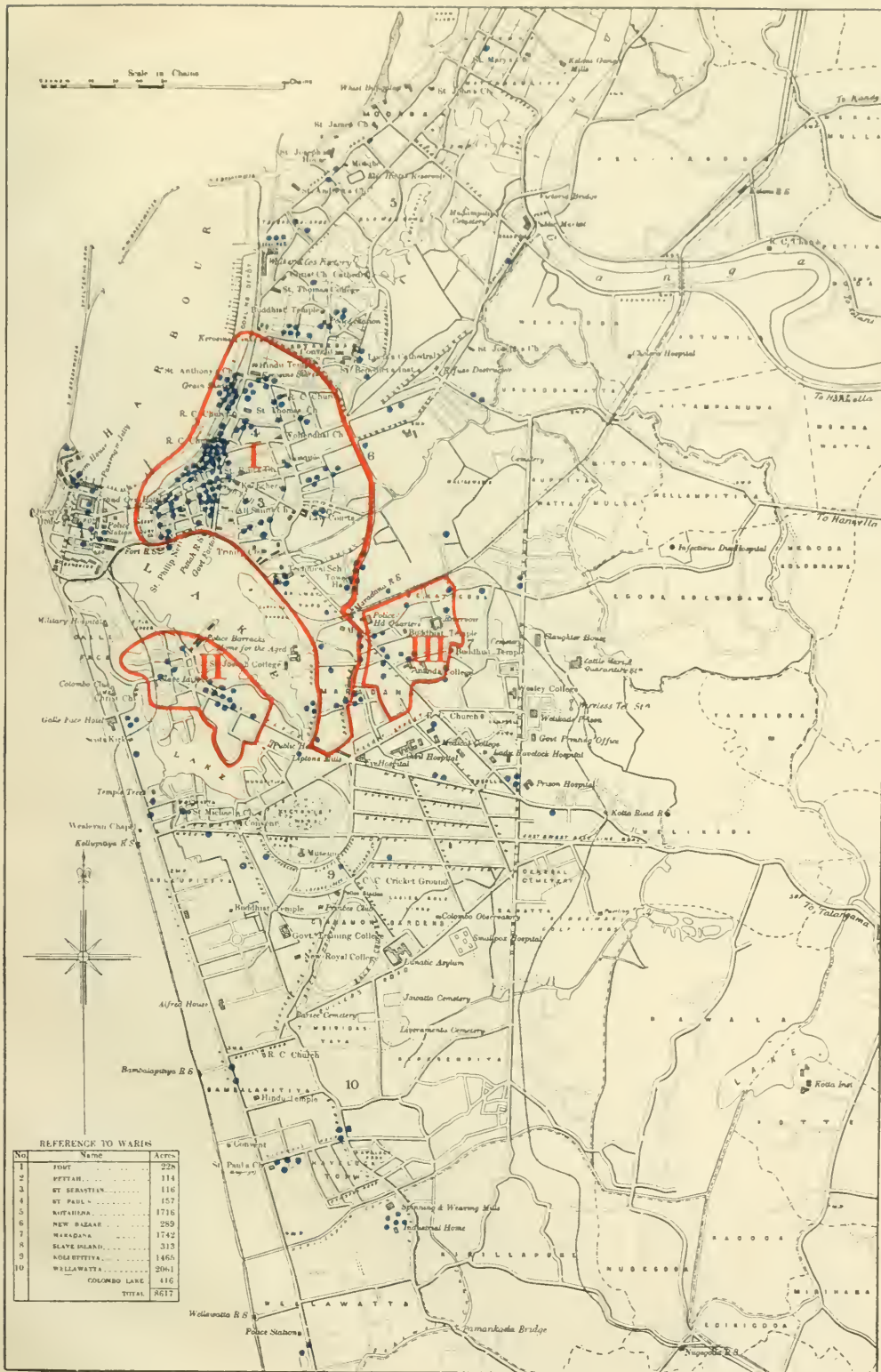
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Human Plague in Colombo, 1914

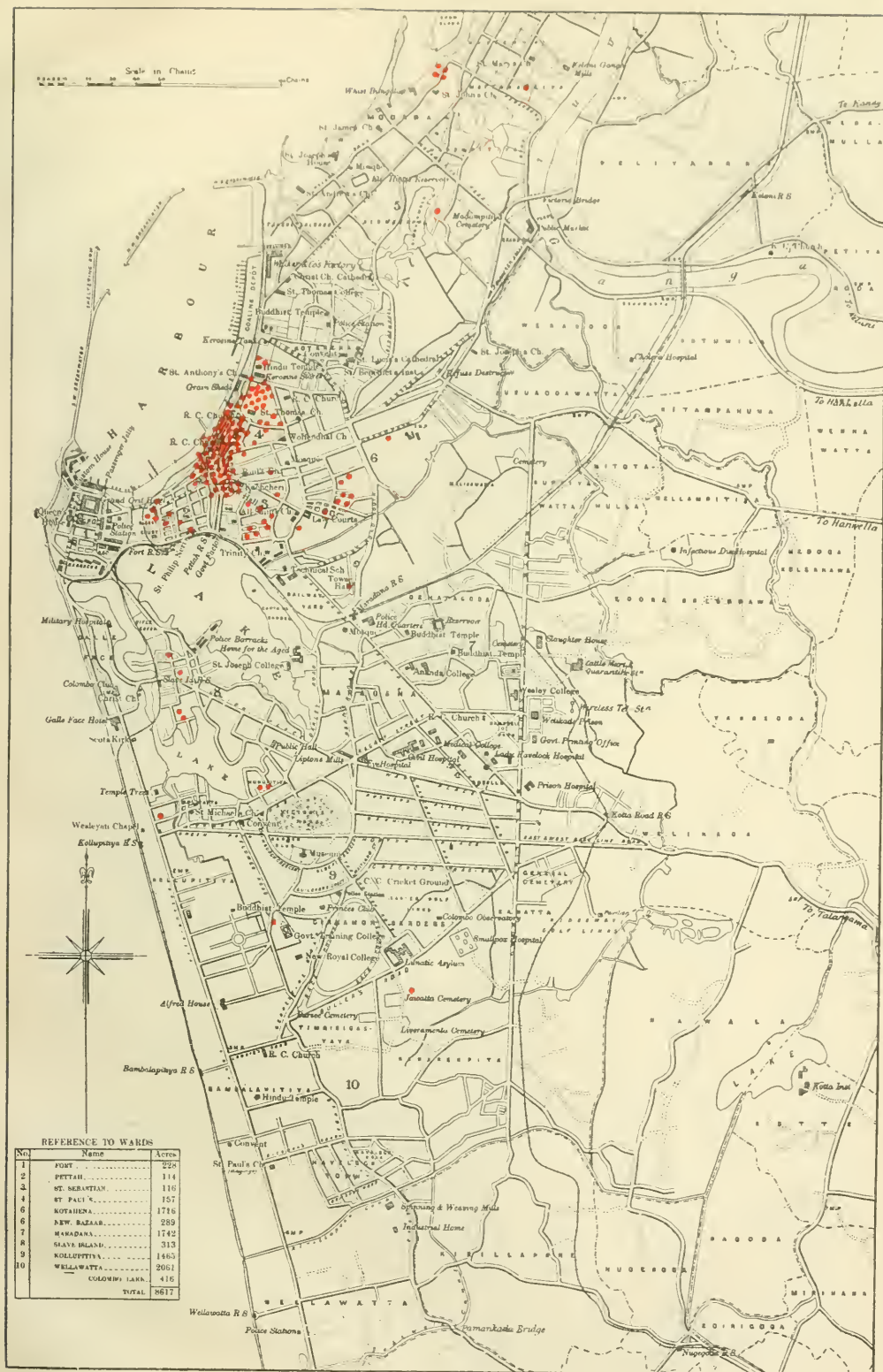






Rat Plague in Colombo, 1914

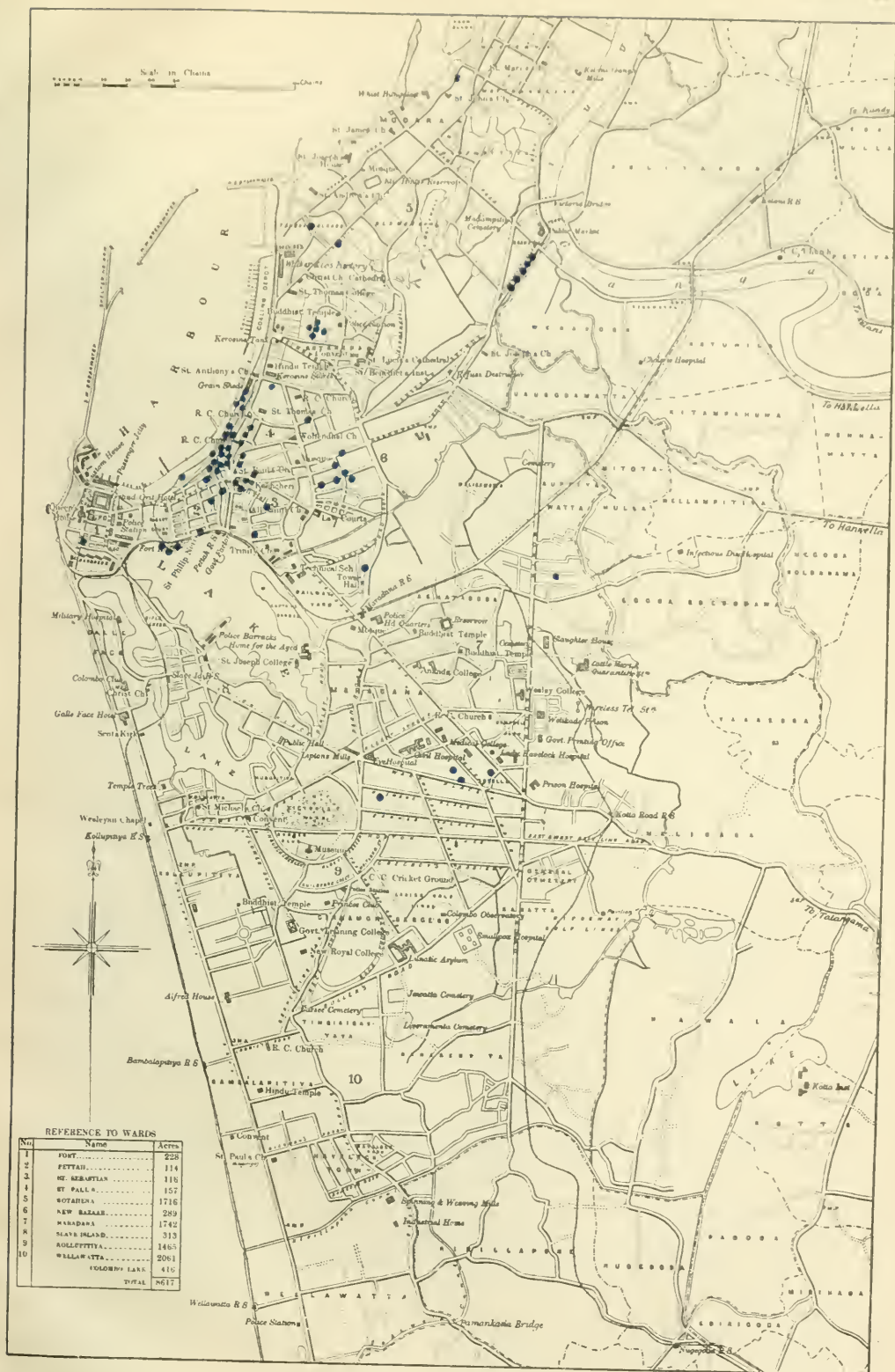




Human Plague in Colombo, 1915







Rat Plague in Colombo, 1915



## OBSERVATIONS ON EAST AFRICAN BACILLARY DYSENTERY.

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### INTRODUCTORY.

EAST African dysentery has ceased to be of merely local importance. With troops here from nearly all parts of the Empire carriers are almost certain to take infections acquired here to other places. I am emboldened therefore to put these observations on record, because, with one exception, so far as I am aware no observations have been published on bacillary dysentery in Eastern Africa. I am the more encouraged to do so because I have to record the common occurrence of an organism which has not hitherto been recognised to be a frequent cause of dysentery, viz. Morgan's bacillus.

Manteufel, writing upon an outbreak of dysentery at Dar-es-Salaam, in German East Africa, states that he was the first to demonstrate the occurrence of *B. dysenteriae* in that colony. In 161 cases investigated he found 45 to be bacillary, 9 amoebic, and 107 negative. Of the 45 bacillary cases 15 were positive both by agglutination and by culture from the stools; 21 positive by agglutination, negative by culture; and 9 positive by culture, negative by agglutination.

Of 16 strains isolated and tested 2 appeared to be identical with *B. Flexner* and 7 with *Bac. Y*. No strains corresponded with *B. Shiga* but several curious anomalous varieties were found.

In the *Nairobi Laboratory Report* for Jan.-June, 1914 (vol. v, Part 1), I published a preliminary note on 5 cases. These five and one other are included in this paper although their investigation was incomplete.

In the *Annual Medical Report of the Protectorate* for 1912 it is stated that in this country dysentery invariably depends upon the *Amoeba*. This statement, I fancy, was based upon insufficient evidence. Be that as it may the general impression here now is that amebic dysentery during the past few years has become less common while dysentery presumably of bacillary origin (the opinion being based mainly on negative evidence—absence of amoebae from the stools) has become increasingly frequent.

During the campaign it has only been possible to have a very small proportion of the dysentery cases examined bacteriologically or even microscopically, but of those examined comparatively few proved to be amoebic. Tentative emetine treatment in undifferentiated cases has also failed to be of benefit in most instances. Other protozoa (e.g. *Lamblia*) occur occasionally, but they may be ruled out as comparatively negligible factors in the causation of dysentery. Fine suspended particles of mica in the water of certain districts (especially the Tsavo river) may account for some cases of a chronic nature but I think it may be taken as reasonably certain that most of the dysentery here at present is bacillary in origin.

#### SOURCE OF STRAINS.

56 cases in all (A to F and 1 to 50) are included in this paper. Six of these (A to F) date back to June, 1914. The study of these was unfinished when I was called out for Field Service and as the cultures had all died out before my return to the Laboratory they must remain incomplete. The remaining 50 cases were obtained between January and August, 1916.

All 56 cases with the exception of case C, a European lady suspected of being a typhoid carrier, were clinically dysentery, the stools containing blood, pus, and mucus. They were of varying severity, some slight but most of them severe. In all cases save two the presence of amoebae was excluded by microscopic examination, but it is possible that in some instances amoebae may have been missed or that the bacillary infection may have been secondary to an amoebic one. In two cases amoebae were found. One of these proved negative for dysentery bacilli. In the other (Case 44) no amoebae were found at first, but they were present on a later examination.

Incidentally I might note here that the macroscopic appearance of the stools seems to be of no value whatsoever in determining whether



the disease is amoebic or otherwise in origin. Microscopically however if the stools are examined within the first day or two of the disease, a marked paucity of bacteria suggests the presence of amoebae even although they may not actually be found, and in such circumstances a trial of emetine is recommended. In later stages of the disease this difference between amoebic and bacillary cases does not hold good.

Most of the cultures were made from stools, a few from the scrapings of ulcers in the large intestine *post-mortem*.

Of these 56 cases 39 yielded non-lactose fermenters on MacConkey plates, and from these 39 dysentery-like bacilli were obtained in 20. These 20 cases were as follows. Two other cases (21 and 21) are also included in the list.

- B. European infant. Severe attack but recovered. Stool.
- C. European lady. Suspected typhoid carrier. Stool.
- D. European male. Mild case. Stool.
- 1. Kavirondo. Carrier Corps porter. Post-mortem.
- 2. Kavirondo. Carrier Corps porter. Post-mortem.
- 5. European. Sergeant. L.N. Lanes. Stool.
- 8. Kavirondo. Carrier Corps porter. Stool. Case died later.
- 12. Indian baby. Stool. Case died later.
- 15. Kikuyu. Carrier Corps porter. Stool. Case died later.
- 17. European. Private. L.N. Lanes. Stool.
- 19. European. Private. L.N. Lanes. Stool.
- 21. Kavirondo. Stool.
- 23. Indian child. Stool.
- 24. European lady. Stool. Chronic case. Acute attack about a year previously. Passing pus and mucus ever since.
- 28. Swahili houseboy. Stool. Ran away from hospital. Probably recovered.
- 33. European child. Stool. Recovered.
- 34. Kavirondo. Carrier Corps porter. Stool. Case died later.
- 38. M'Kamba. Carrier Corps porter. Stool. Recovered.
- 39. Kavirondo. Carrier Corps porter. Stool. Recovered.
- 44. Baganda. Stool. Case still severe after three months in hospital.
- 45. Baganda. Stool. Case died later.
- 50. Kavirondo. Carrier Corps porter. Stool. Recovered.

That only 20 of the 56 cases (35.7 %) yielded dysentery-like bacilli is probably partly accounted for by shortage of laboratory materials severely limiting the number of colonies that could be examined from each plate. Intermittent occurrence or actual absence of dysentery bacilli may account for others. Various observers report similar findings, *e.g.* Manteufel, quoted above; Denier, from 329 cases found dysentery bacilli in 30.48 % of dysenteric stools and in 17.72 % of

TABLE I.

Culture No.	Lactose	Gelatin	Mannite	Glucose	Saccharose	Dulcitol	Maltose	Salicin	Litmus milk			Indol	Motility	Laevulose	Dextrin	Inulin	Adonite	Agglutinations			Approximate Grouping
									1 day	3 days	15 days							Flexner	Shiga	Y	
B	-	-	-	-	-	-	-	-	A	Alk	-	+	-	-	-	-	-	-	-	-	Patient's Serum
C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Polyvalent "D 25"
D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Y
1 A	-	-	-	-	-	-	-	-	0	A	A	-	-	A	-	-	-	-	-	-	Shiga
1 B	-	-	-	-	-	-	-	-	0	0	A	-	+	A	-	-	-	-	-	-	Flexner-Y
3 A	-	-	-	-	-	-	-	-	0	0	clot	-	+	AG	-	-	-	-	-	-	Flexner-Y
3 B	-	-	-	-	-	-	-	-	0	0	clot	-	+	AG	-	-	-	-	-	-	Shiga ?
5 B	-	-	-	-	-	-	-	-	0	0	Alk	+	+	AG	-	-	-	+	trace	-	Morgan
8 A	-	-	-	-	-	-	-	-	0	0	Alk	+	+	AG	-	-	-	+	slight	+	Morgan
8 C	-	-	-	-	-	-	-	-	0	0	Alk	+	+	AG	-	-	-	-	-	-	Morgan
10	-	-	-	-	-	-	-	-	0	0	A	+	-	AG	-	-	-	-	-	-	Morgan ?
11	-	-	-	-	-	-	-	-	0	0	clot	+	+	Alk (?)	-	-	-	+	+	+	?
12	-	-	-	-	-	-	-	-	A	Alk	Alk	-	+	A slight	-	-	-	+	+	+	Shiga
15 A	-	-	-	-	-	-	-	-	A	Alk	Alk	+	-	A	-	-	-	+	slight	-	Flexner-Y
15 B	-	-	-	-	-	-	-	-	A	Alk	Alk	+	-	A	-	-	-	-	-	-	Flexner-Y
17	-	-	-	-	-	-	-	-	0	0	A	+	-	A	-	-	-	-	-	-	Flexner-Y

slight late

slight

slight

19 A	-	-	AG	AG	-	-	-	AG	-	+	-	AG	A slight	-	-	?
19 B	-	-	-	AG	-	-	-	-	AG	+	+	AG	-	-	-	Morgan
21	-	-	AG	AG	-	AG	A	0	Alk	0	0	A slight	-	-	-	Bac. enteritidis
22 A	-	-	-	-	-	-	-	0	Alk	Alk	-	-	-	-	-	?
22 B	-	-	-	A	-	A (?)	late	0	Alk	A	-	A	-	-	+	?
23	-	-	-	A	-	-	-	Alk	A	A	-	A	-	-	+	Shiga
24 A	-	-	AG	A	-	AG	AG	A	0	+	+	-	A	-	-	Bac. enteritidis
24 B	-	-	AG	AG	-	AG	AG	A	0	+	+	-	AG	A	-	Bac. enteritidis
28 B	-	-	-	AG	-	-	AG	0	Alk	Alk	+	+	AG	-	-	Morgan
28 C	-	-	-	AG	-	-	-	Alk	Alk	Alk	+	-	AG	-	-	Morgan
33 B	-	-	-	AG	-	-	-	Alk	Alk	Alk	+	-	AG	-	+	Morgan
34 A	-	-	-	AG	-	-	-	0	Alk	Alk	+	+	AG	-	-	Morgan
38 A	-	-	-	AG	-	-	-	Alk	Alk	Alk	+	+	AG	-	-	Morgan
38 B	-	-	-	AG	-	-	-	Alk	Alk	Alk	+	+	AG	-	-	Morgan
39 A	-	-	-	A	-	-	-	0	Alk	Alk	-	-	A	-	-	Shiga
44 A	-	-	-	AG	-	-	AG slight	Alk	Alk	Clot	+	+	AG	-	+	Morgan
44 B	-	-	-	A	-	-	-	Alk	Alk	Alk	+	+	AG	-	-	Morgan
45 A	-	-	-	AG	-	-	-	Alk	Alk	Alk	+	+	AG	-	-	Morgan
45 B	-	-	-	A	-	-	AG	Alk	Alk	A	+	+	AG	-	-	Morgan
50 B	-	-	-	A	-	-	-	0	Alk	Alk	-	-	A	-	+	Shiga

diarrhoeic stools; in 197 of Heffernan's cases dysentery bacilli were found in 50 %, amoebae in only 5 cases. Tribondeau and Fichet in 217 cases from the Dardanelles found amoebae in 10 cases and dysentery bacilli in 38 (17 %), but in this instance it must be noted that their cases were all past the acute stage and in process of recovery.

#### TECHNIQUE.

The method of procedure employed was based on those recommended by Henderson Smith and by Ledingham and Penfold. A loopful of mucus from the stool or bowel was shaken up in a tube of broth. After standing for about an hour a loopful of broth taken from the top layers of the tube was plated on MacConkey's bile-salt neutral-red lactose agar and incubated at 37° C. for at least 18 hours. Colourless colonies were then picked off, inoculated individually into broth, incubated again for a few hours or overnight, and then inoculated again into mannite-peptone water and on to agar. Examination for motility in the young broth culture and the behaviour towards mannite as a rule decided whether we were to proceed further with the examination or not. Further inoculations into gelatin and the various sugars etc. available were made either direct from the agar slope or from a broth sub-cultured from it.

Table I gives the characters of 36 organisms isolated.

Agglutination was tested by the microscopic method, four hours being allowed before finally deciding whether it was positive or negative. The anti-Shiga, anti-Flexner, and anti-Y sera were kindly supplied me by the Lister Institute, also the "D 25" serum, this being a polyvalent anti-dysenteric serum prepared for therapeutic purposes. Further details regarding degree of agglutinability will be found in the subsequent discussion of results.

Blanks in the table mean "not tested."

#### *Grouping of these 36 strains.*

Cultures 1 B, 10, 11, 19 A, 22 A, and 22 B may I think be dismissed from further consideration as not corresponding even approximately with any recognised pathogenic organism.



ORGANISMS RESEMBLING *B. Shiga*.

Six cases. Cultures Nos. B, 1 A, 12, 23, 39, and 50 B.

B was insufficiently tested. 1 A and 23 agree in their cultural reactions with the typical *B. Shiga* except in their behaviour towards milk. 1 A is not agglutinated by anti-Shiga, Flexner, or Y sera in a dilution of 1 in 200 while 23 is agglutinated to practically an equal extent by all three in that dilution. Remlinger and Dumas (1915) have recently recorded from the Argonne an organism closely related to *B. Shiga* but not agglutinated by Shiga serum. Generally speaking however the Shiga bacillus has been found to occur in many parts of the world very true to type and to remain so even after repeated sub-cultivation (although variations do then occur; see, for instance, Barber, also Hallenberger). My observations are only sufficient to enable me to put these organisms on record as resembling but not identical with *B. Shiga*.

No. 12 agrees with the typical Shiga organism very closely. It is agglutinated by Shiga serum very markedly, slightly by Flexner serum, and not at all by Y (all in dilutions of 1 in 200). Its character as regards motility however is curious. Bacilli of this class frequently show oscillatory and turning movements which make one doubtful at times whether one is dealing with a feebly motile organism or not, but the first two generations in broth (off the original MacConkey plate) of culture No. 12 left one in no doubt. It was an actively motile organism. Subsequent generations (young broth cultures) taken from an agar slope, however, appear to be non-motile, or, at most, to show nothing more than ordinary oscillatory and turning movements. Whether this organism therefore should or should not be accepted as a *B. Shiga* is also doubtful, although anyone working with it as it is now would, I think, accept it as such, unless possibly further tests which I am not in a position to apply, showed further discrepancies.

Nos. 39 and 50 B correspond with the typical *B. Shiga* both by fermentation and agglutination tests. As regards agglutination 39 reacts slightly with "D 25" 1 in 30, well with anti-Shiga 1 in 100 and slightly 1 in 500, also well with the patient's own serum diluted 1 in 100.

No. 50 agglutinates well with the polyvalent serum 1 in 30, very well with anti-Shiga 1 in 100 but not 1 in 500, also well with the patient's serum 1 in 50.

ORGANISMS RESEMBLING THE *FLEXNER-Y* GROUP.

Four cases. Cultures C, D, 15 A and B, and 17.

C and D were insufficiently tested but it might be noted that in case C (a suspected typhoid carrier) the organism agglutinated with the patient's own serum markedly in a 1 in 90 dilution, slightly with 1 in 180. The patient's serum also agglutinated *B. typhosus* markedly with a 1 in 180 dilution.

Nos. 15 A and B correspond with the typical *B. Flexner* but ferment dulcitate additionally. They do not agglutinate with either Shiga, Flexner, or Y sera 1 in 200.

No. 17 is less typical. It resembles the *Bac. Y* except in fermenting saccharose and clotting milk. In these two respects it resembles the *B. Strong* but differs from that organism in fermenting dextrin and in not fermenting dulcitate and maltose. It also does not agglutinate with Shiga, Flexner, or Y sera 1 in 200.

Great variability has been recorded amongst this group both as regards fermentation and agglutinability (see especially Morgan, also Gettings and Barber), but I think that at least the strains from case No. 15 have sufficient claim to be regarded as belonging to the mannite-fermenting group of dysentery bacilli. Whether such organisms should be labelled "Para" or "Pseudo" dysentery bacilli and be regarded as distinct species, or whether they are to be looked upon as merely varieties of one common type is part of a very large question which is beyond the scope of this paper.

ORGANISMS RESEMBLING *MORGAN'S BACILLUS*.

Ten cases. Cultures Nos. 3 A and B, 5 B, 8 A and C, 19 B, 28 B and C, 33, 34, 38 A and B, 44 A and B, and 45 A and B.

Nos. 5 B, 8 A and C, 34 A, 38 A and B, 44 B, and 45 A agree completely in their cultural and fermentation reactions with Morgan's No. 1 bacillus.

Nos. 38 A and 44 B are, however, only feebly motile, not actively motile as the typical Morgan's No. 1 is stated to be.

No. 5 agglutinated slightly with Flexner serum but not with Shiga or Y (dilution 1 in 200).

Nos. 8 A and 8 C did not agglutinate with these sera in 1 in 200 dilution.

No. 34 A did not agglutinate with polyvalent serum 1 in 30.

Nos. 38 A and 38 B agglutinated slightly with polyvalent serum 1 in 30, with the patient's serum well 1 in 50, and slightly 1 in 100, not with Shiga serum 1 in 100.

No. 44 B did not agglutinate with polyvalent serum 1 in 30 nor with the patient's serum 1 in 50.

No. 45 A did not agglutinate with polyvalent serum 1 in 30 but gave a slight reaction with the patient's serum 1 in 50 although not 1 in 100.

Nos. 19 B, 28 C, and 33 B correspond in their characters with Morgan's No. 1 bacillus except that they are non-motile. In this connection it might be noted that Tribondeau and Fichet in their 13 cases found 7 actively motile, 4 feebly, and 2 non-motile. 28 C did not agglutinate with polyvalent serum 1 in 30; 33 B did slightly. No other agglutinations were tried with this lot.

Nos. 3 A and B, 28 B, 44 A, and 45 B present characters intermediate between Morgan's bacillus and Bowman's "Bac. S," although varying amongst themselves. Bowman's bacillus, said to be a common cause of dysentery, especially amongst children in the Philippines, is described in the abstract of Musgrave and Sison's paper in the *Bull. de l'Inst. Pasteur* as being "très voisin du bacille Morgan I"; in the abstract of Bowman's original paper there are at least three characters mentioned which distinguish it from that organism, viz. fermentation of maltose, non-production of indol, and clotting of milk. The following table (Table II) shows the variations in this series of strains.

TABLE II.

		Maltose	Milk	Indol	Motility
"Typical Morgan I" ...	...	-	Alk.	+	active
"Typical Bac. S" ...	...	AG	Clot	-	active
3 A and 3 B ...	...	-	Clot	-	active
28 B ...	...	AG	Alk.	+	slight
44 A and 45 B ...	...	AG	Clot	+	slight

45 B also differed from Morgan's and Bowman's organisms in forming acid only without gas from glucose.

As regards agglutinations 3 B gave a very slight reaction with anti-Shiga serum 1 in 200, not with Flexner or Y in that dilution. 3 A gave no reaction with those three.

No. 28 B gave no reaction with polyvalent serum 1 in 30.

Nos. 44 A and 45 B agglutinated slightly with patient's serum 1 in 50 but not 1 in 100, nor with polyvalent serum 1 in 30.

Morgan's bacillus was originally found and described from cases of summer diarrhoea amongst children in England. I am unable to

refer to the original descriptions of the various strains by Morgan and by Morgan and Ledingham, but have taken the characters of Morgan's No. 1 Bacillus as summarised by Muir and Ritellie and Henderson Smith to be correct. Possibly some of my atypical strains correspond with some of Morgan's varieties other than No. 1.

The occurrence of Morgan's Bacillus in cases of what clinically appears to be dysentery has been recorded by Gettings and by Orr in asylum dysentery in England. In India, Morison and Chitre from 95 cases of diarrhoea and dysentery studied some 1500 organisms. Morgan's No. 1 bacillus was found in 16 cases, *B. Shiga* in 16, and *B. Flexner* in 10. Ten other cases also showed organisms having fermentation reactions approaching those of recognised dysentery bacilli.

Morgan's bacillus would also appear, according to Musgrave and Sison, to be a common cause of dysentery, especially amongst children, in the Philippines, where also occurs *Bowman's Bac. S.*

More recently Tribondeau and Fichet record 13 cases of Morgan's bacillus dysentery from the Dardanelles and refer to a small epidemic of 5 cases amongst troops at Toulon. They produce good evidence for their belief that the presence of Morgan's bacillus is not fortuitous in those cases but that it is the active pathogenic agent and must be added to the growing list of dysentery bacilli. Of their 13 cases there were three types; 7 corresponding with Morgan's No. 1, 1 with Morgan's No. 3, and 5 with Morgan's No. 29.

They found agglutination and deviation of complement, as tested with the patient's serum, entirely negative. In this connection it might be noted that Ten Broeck and Norbury state that agglutinins are not found in the blood of cases from which the bacillus is isolated, but Ledingham in reviewing their paper states that this is not correct and refers to his work published in a communication by Morgan and himself (*Proc. Roy. Soc. of Med., Epidemiol. Sect.*, March, 1909). In the six of my cases in which agglutination with the patient's own serum was tested it will be noted that evidence of slight agglutinating qualities was found in five.

#### ORGANISMS RELATED TO THE GAERTNER-PARATYPHOID GROUP.

Nos. 21, 24 A, and 24 B show affinities with this group so far as their fermentation reactions go but they are all three non-motile and produce indol abundantly. Their non-motility would apparently make them more closely allied to the *Bac. enteritidis B.*, an organism which however I only know from the table given by Castellani and Chalmers.



## RELATIVE PATHOGENICITY OF THE DYSENTERY BACILLI.

The cases are too few in number to generalise upon the question whether the Shiga bacillus produces a more severe type of the disease than other varieties, but it may be noted that of the seven fatal cases in my series two yielded the Shiga bacillus, one a Flexner-like organism, and four Morgan's bacillus.

Kuenen states that pseudo-dysentery bacilli (meaning types other than Shiga) as a rule set up a less severe form of the disease in Sumatra, although they may nevertheless cause a high percentage of deaths.

Hallenberger writes that in the tropics it does not always hold good that the *B. Shiga* produces a more serious illness than the mannite-fermenting varieties of *B. dysenteriae*. The opposite may occur.

## SERUM TREATMENT.

Through the kindness of the Lister Institute I received a large quantity of polyvalent anti-dysenteric serum for trial treatment. Three strains (D 23, D 24, and D 25) were supplied me. Capt. Thompson, E.A.M.S., gave me an opportunity of testing these sera on cases in the African Base Military Hospital, Nairobi. A large number of cases were available and the treatment was given a thorough trial on cases which microscopic examination showed to be non-amoebic. Only severe cases were chosen as so many of the milder cases were recovering under ordinary medicinal treatment. Some severe cases were at the same time not given serum so as to serve as controls. All cases received similar medicinal and dietetic treatment.

The serum was employed in doses of from 20 c.c. to 100 c.c., in most cases the amount given being nearer the upper limit than the lower. The dose was injected into the subcutaneous tissues of the flanks and was as a rule repeated daily.

I regret to have to report that in no single case did the serum appear to have the slightest beneficial effect. The great majority of the cases in which it was employed died. A few recovered but not a larger proportion than amongst those who did not receive serum.

Dr R. W. Burkitt allows me to state that he gave a polyvalent serum a considerable trial on Europeans in his private practice in Nairobi but that he had given it up as he had failed to get any good results. He injected subcutaneously 100 c.c. as a general rule in adults, at the earliest possible stage of the attack.

The failure to get benefit by polyvalent serum treatment here is very disappointing seeing that it has yielded good results in various other places.

Willmore and Savage quote Vaillard and Dopter as still insisting on the value of a monovalent Shiga serum as efficient in the treatment of all bacillary dysenteries, but state that these writers appear to stand practically alone in this contention. Their own experience at El Tor (where Shiga cases are rare) was all against this view, but with polyvalent serum they obtained very good results. Their case mortality rate in 1911-1912 when no serum was available being 70 %, falling to 12 % in 1912-1913 when polyvalent serum treatment was employed. The rate for the earlier period was probably abnormally high but they adduce good evidence to show that the fall coincident with the introduction of serum treatment was not entirely fortuitous.

Kuenen reports good results with Shiga-Kruse serum in epidemic (Shiga) dysentery and states that the serum appears to act equally well in cases of pseudo-dysentery.

Bahr in Fiji, using the Lister Institute polyvalent serum, had only two deaths in 106 cases (1.8 %), whereas in 53 cases treated without serum the case mortality rate was 13.2 %.

The same observer in abstracting the article by Musgrave and Sison, who found polyvalent serum fail, states that their experience "is entirely opposed to that of other investigators of the disease." Whether this be so or not I am not sufficiently in touch with recent literature to say, but Remlinger and Dumas working in the Argonne region, with cases due mainly to a *Y* bacillus, report the inefficacy of both anti-Shiga serum and polyvalent anti-Shiga-Flexner-*Y* serum.

Musgrave and Sison consider that for practical purposes, in places where the means of identifying the infecting micro-organism are not available, treatment by polyvalent serum fails. They ascribe this to the important part played by other organisms such as staphylococci and colon bacilli in producing the inflammation of the intestinal mucosa.

What the reasons for the failure of serum treatment here may be is as yet uncertain. One probable factor is the frequency of Morgan's bacillus as the cause of the dysentery, as I fancy this organism is not employed in the preparation of the polyvalent serum. It may be also that the other strains of dysentery bacilli found here are too remotely allied to those from which the sera were prepared.

Once let the dysentery bacilli get a good start I am of opinion that much may be said for the view that common intestinal organisms play

an important secondary rôle. Clinically it is frequently noted here that cases apparently get over the acute attack, but that they do not pull round and sooner or later die, although their motions have ceased for some time to have a dysenteric character. Seven cases of this sort were amongst those I investigated. Post-mortem examination of four showed extensive ulceration of the large intestine, in two instances there being very little mucosa left throughout its whole length. Cultures in four of the seven cases failed to yield any dysentery bacilli; in the other three they were found either ante- or post-mortem.

Such cases would suggest that the other bowel organisms may be responsible in the later stages of the disease for the destruction of the mucosa; death following on absorption of faecal or bacterial poisons through the extensive raw surface.

The recent view advanced by Remlinger and Dumas that death is in many instances due to a supra-renal insufficiency and the results recorded by Parhon with treatment by adrenalin are suggestive. The treatment by adrenalin is being given a trial here.

Vaccine treatment with mixed Shiga-Morgan vaccines is also being tried both therapeutically and as a prophylactic measure.

Specimens of these various cultures have been sent to the Lister Institute, London.

#### SUMMARY.

Fifty-six cases examined for Dysentery bacilli. All save one were clinically dysentery, and all save two were negative for amoebae and other protozoa.

Twenty cases (35·7 %) yielded dysentery-like bacilli.

From these twenty cases thirty-six organisms were isolated and studied. Of these—

Six corresponded with no familiar pathogenic organism.

Six resembled *B. Shiga*. Two agreed completely both by fermentation and agglutination. One also gave typical fermentation and agglutination results but was at first a motile organism, although in later generations it became non-motile. The other three are less definitely Shiga bacilli.

Five were mannite-fermenting organisms but only one agreed very closely in its fermentation reactions with *B. Flexner* and it was not agglutinated by Flexner serum.

Sixteen cultures from ten separate cases corresponded closely with Morgan's bacillus.

Eight of these agreed completely in their characters with *Morgan's No. 1* bacillus.

Three differed only from *Morgan's No. 1* in being non-motile.

Five presented characters intermediate between those of *Morgan's No. 1* and Bowman's "*Bac. S.*"

Of six cases yielding Morgan's bacillus, agglutination with the patient's own serum was positive in five.

Three cultures showed affinities with the *Bac. enteritidis*.

Polyvalent anti-dysenteric serum treatment tried on a considerable number of cases was a complete failure.

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## ON FACTORS LIMITING THE EXTENT OF THE CONCENTRATION OF ANTITOXIC SERA BY THE FRACTIONAL PRECIPITATION METHODS AT PRESENT EMPLOYED.

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THE use of concentrated antitoxic sera in the treatment of diphtheria and tetanus has been generally recommended with a view to minimising the troubles of serum sickness. For, during the process of concentration of sera, there is a removal of the proteins not associated with antitoxin. It is thus possible to administer large doses of antitoxin with far less discomfort to the patient and with the introduction of a considerably smaller amount of the proteins of horse serum than if the original unconcentrated sera had been used.

The aim of any concentration process is therefore the preparation, on a commercial scale for therapeutic uses, of antitoxic sera which shall contain a minimum amount of attendant protein.

In a previous communication (Homer, 1916) it has been shown that, in the method recently adopted in this laboratory for the concentration of sera, a removal of from 65 to 70 % of the total proteins of the original serum is effected with a consequent increased potency eight to nine times that of the original serum.

The new method thus presented a step towards the desired goal, for by previous methods there had been a removal of about 40 % of the total proteins with a consequent increased potency of from four to five times. From experimental observations it was evident that the heat denaturation<sup>1</sup> of the serum proteins was the primary factor influencing the removal of protein beyond that obtained by the methods previously in vogue.

<sup>1</sup> Throughout this paper the term "heat denaturation" is used as a convenient term for the increased precipitability of the serum proteins by 30 % of saturation with ammonium sulphate induced by the prolonged heating of serum.

Some definite relationship thus exists between the extent of the removal of unnecessary protein from the serum and the degree of concentration of antitoxin in the final product. Therefore the question arose as to whether the heat denaturation of the proteins could not be induced to an extent greater than that obtained in our new process so as to yield a final product in which the percentage of protein had been still further reduced.

In the course of experimental work I have ascertained that the heat denaturation of the serum proteins is considerably influenced by the presence of acid and of electrolytes. But, as was anticipated, the presence of acid during the prolonged heating of sera, even at 57–58° C., under some conditions has a deleterious effect on the antitoxin: a loss of as much as 50 % of the antitoxin has been measured. On the other hand, as is shown in Table I, in the presence of the electrolytes employed there was no appreciable destruction of antitoxin at a temperature of 57–58° C. Heating the serum in presence of 30 % of ammonium sulphate to a temperature of 61° and 63° for 2 hours resulted in the destruction of 20 % of the antitoxin. With 10 to 20 % of sodium chloride there was no loss after 2½ hours' heating at 62° C. But, when the serum was saturated with salt and heated to 62° for 2½ hours the mixture coagulated; the residue after dilution with an equal bulk of 1 % saline, was tested for its antitoxin value and showed a loss of about 50 %.

In view of these results the use of sodium chloride as the denaturing agent was more carefully investigated.

Serum or plasma was diluted with one-third its volume of water, definite amounts of the electrolyte were added and the diluted serum was then heated to a temperature of 57–58° C. for periods of 7 to 8 hours. The heated liquid was made 30 % of saturation with ammonium sulphate, the temperature raised just to 60° C., and the mixture cooled to 50° C. and filtered. The precipitate (*First Fraction Precipitate*) was washed with 30 % saturated ammonium sulphate and the washings added to the main bulk of the filtrate. The filtrate and washings were then brought up to 50 % of saturation with ammonium sulphate and filtered. The precipitate (*Second Fraction Precipitate*) was pressed and dialysed. The residue from the dialysis of this precipitate was made 0.35 % with cresylic acid and the necessary amount of salt was added (*Final Product*).

The protein and the antitoxic contents of the final products were estimated in the usual way and were compared with those of the original serum.

TABLE I.

*The loss of antitoxic units during the prolonged heating of antitoxic sera to which electrolytes have been added.*

*In each case the serum or plasma was diluted with one-third its volume of water previous to the addition of the electrolyte.*

	Electrolytes added to the diluted plasma	Temperature to which the plasma, after the addition of the electrolyte, was heated	Duration of the time of heating at the specified temperature	Unitage of the original serum	Percentage loss of antitoxic units during the heating process
<b>Tetanus:</b>					
Adelaide 1/6/16	1½ % NaCl	57-58° C.	8 hours	150	negligible
Adelaide 1/6/16	4 "	"	8 "	150	"
<b>Diphtheria:</b>					
△ R. 41	2 "	"	8 "	250	"
△ R. 42	5 "	"	4 "	250	"
△ R. 40	2 "	"	8 "	500	"
Lille 26/6/16	8 "	"	8 "	150	less than 10 % *
Lille 26/6/16	20 "	"	8 "	150	about 10 %
Ethel 2/5/16	20 "	"	8 "	350	negligible
Lille 26/6/16	Saturated with NaCl	"	8 "	150	"
Ethel 2/5/16	Saturated with NaCl	"	8 "	350	"
Lille 26/6/16	2 % NaCl + 30 % AM <sub>2</sub> SO <sub>4</sub>	"	8 "	150	"
Ethel 2/5/16	2 % NaCl + 30 % AM <sub>2</sub> SO <sub>4</sub>	61°	8 "	350	20 %
Ethel 2/5/16	2 % NaCl + 30 % AM <sub>2</sub> SO <sub>4</sub>	63°	8 "	350	"
Ethel 10/10/16	10 % NaCl	62°	2½ "	300	negligible
Ethel 10/10/16	20 % NaCl	"	2½ "	300	"
Ethel 10/10/16	Sat. NaCl	"	2½ "	300	about 50 %

\* Not tested out.



TABLE II a.

*The precipitation of serum proteins by the addition of sodium chloride to plasma (1) previously diluted with half its volume of water, and (2) previously diluted with half its volume of water and made 30 % of saturation with ammonium sulphate.*

	(1)	(2)
Percentage of NaCl added to the diluted plasma.	Percentage of Residual Protein in solution in the plasma after the addition of the various percentages of NaCl. Expressed in terms of the original plasma	Percentage of Residual Protein in solution after making the salted plasma 30% saturation with ammonium sulphate. Expressed in terms of the original plasma
0	7.70	6.20
1	7.68	6.20
2	7.68	5.50
3	7.65	5.38
4	7.37	5.37
5	7.32	5.32
6	7.32	5.30
7	7.32	4.15
8	7.19	3.75
9	6.99	3.70
10	6.86	3.70

TABLE II b.

*Showing the influence exercised by sodium chloride on the precipitation of antitoxin along with the First Fraction Precipitate both (1) before and (2) after the prolonged heating of the serum.*

Serum	Unitage	Percentage of salt added to the diluted plasma	Unitage of the filtrate from the First Fraction Precipitate
(1) <i>The unheated plasma:</i>			
Adelaide 25/5/16 Tetanus	150	4	150 (guinea pig used in testing the serum for this unitage died in 90 hours)
Adelaide 25/5/16 „	150	7	150 (guinea pig used in testing the serum for this unitage died within 48 hours)
(2) <i>The heated plasma:</i>			
Adelaide 1/6/16 Tetanus	150	2	145 nearly (guinea pig used in testing the serum for this unitage died in 90 hours)
Adelaide 1/6/16 „	150	4	145 just
Adelaide 1/6/16 „	150	8	100 (guinea pig used in testing the serum for this unitage died within 90 hours)

Where the percentage of sodium chloride added to the serum had been greater than 5 it was found advisable to dilute the heated liquid before the addition of ammonium sulphate for the first precipitation. Otherwise as is shown in Tables IIa and IIb there is an increased precipitation of protein and of antitoxin in the first precipitate both in the unheated and in the heated plasma.

In Table IIIa have been incorporated the results of those concentrations in which the antitoxic units of the original serum were, within the limits of experimental error, fully accounted for in the final product.

In Table IIIb have been embodied the results of concentrations in which, although there had been no loss of antitoxin during the heating process, the final product showed an appreciable loss of antitoxic units.

A further investigation, however, demonstrated that in the concentrations thus studied the missing antitoxin had been brought down in the First Fraction Precipitate. None was detected in the albumin fraction (filtrate from Second Fraction Precipitate). Moreover, the antitoxin thus brought down with the First Fraction Precipitate could not be recovered by washing the precipitate with 30 % saturated ammonium sulphate: for this purpose extraction with saturated salt solution was essential.

A study of the data in Tables IIIa and IIIb reveals the fact that *there is a limit to the extent to which the removal of the serum proteins can be effected without disturbing the precipitation of the antitoxin with the Second Fraction Precipitate.* For, it is apparent that if the percentage removal of the total serum proteins be increased much beyond 80 %, there is a consequent increased precipitation of antitoxin in the First Fraction Precipitate.

It is also obvious from Table IIIa that the order of the increased potency measured in the Final Product depends upon two factors. The one factor is the extent of the denaturation induced during the heating process and its effect on the removal of proteins during the concentration processes. The other factor is the efficacy of the pressing of the Second Fraction Precipitate before dialysis.

From the results thus recorded it will be seen that it has been possible to concentrate sera by as much as 12 times while the percentage protein content of the Final Product has not been greater than 20.

In an experimental concentration in which it was calculated that the potency would be increased by 15 times it was however found to be only  $8\frac{1}{2}$  times that of the original. The antitoxin, thus not present

TABLE III a.

*Showing the percentage of the total antitoxic units of the original serum recovered in the Second Fraction Precipitate in cases where the percentage removal of the proteins of the original serum during the concentration process has not been greater than 80.*

	Unitage of the original serum	Percentage protein content of the original serum	Percentage of electrolytes added to the plasma	Percentage loss of antitoxic units during the heating process	Extent of the heat denaturation	Percentage removal of the total serum proteins during the concentration	Second Fraction Precipitate after Dialysis			Percentage of the total original antitoxic units recovered in the Second Fraction Precipitate
							Percentage protein content	Unitage per c.c.	Increased potency per c.c. over that of the original serum	
Tetanus:										
Adelaide	150	6.16	8% NaCl	negligible	48%	79.0	13.3	1800	12 times (nearly)	95
Diphtheria:										
Lille 26/6/16	150	7.26	8%	"	35%	80.0	14.24	1100	7½	76
Lille 26/6/16	150	7.26	12%	"	40%	71.0	17.3	1600	10½	94
Lille 26/6/16	150	7.26	20%	"	about 10%	70.0	15.0	900	6	80
Smyrna* 7/7/16	600	9.68	7%	not estimated	2%	68.2	20.0	2750	4½	75
Lille 26/6/16	150	7.26	30% Am <sub>2</sub> SO <sub>4</sub>	negligible	20.6%	71.0	10.5	800	5.6	87.5
Tetanus:										
T.R. 35	100	6.61	2% NaCl	not tested†	42.2%	68.9	19.5	900	9	90
T.R. 36	150	7.59	"	†	28.7%	70.0	19.2	1300	8½	80
T.R. 37	200	7.10	"	†	31.3%	60.7	18.04	1300	6½	91
T.R. 38	100	6.60	"	†	32.0%	72.0	15.62	850	8½	85
Diphtheria:										
Δ R. 33	275	—	"	†	—	70.4	17.5	2200	8	92
Δ R. 39	400	8.51	"	†	31.1%	77.3	19.33	3000	7½	80
Δ R. 40	500	7.57	"	negligible	23.5%	60.0	17.0	2500	5	85
Δ R. 42b	250	6.60	5% NaCl	negligible	41.2%	70.0	18.6	2250	8½	86

\* Concentrated by Ranzhaf's 1913 Method.

† We have so often satisfied ourselves that there is no appreciable loss of antitoxin during the heating of serum to which has been added 2% of NaCl, to a temperature of 57–58°C. for a period of 7–8 hours, that we consider it as established that at this stage in the concentration there is no loss of antitoxin.

TABLE IIIb.

*Showing the percentage of the total antitoxic units of the original serum recovered in the Second Fraction Precipitate in cases where the percentage removal of the proteins of the original serum during the concentration process has been greater than 80.*

	Unitage of the original serum	Percentage of protein content of the original serum	Percentage of NaCl added to the diluted plasma	Temperature to which the plasma was heated	Duration of the heating at the specified temperature	Percentage loss of anti-toxic units during the heating process	Extent of the heat denaturation	Percentage of the total proteins removed during the process of concentration	Percentage of original anti-toxin units appearing in the dialysed Second Fraction Precipitate	Presence or absence of anti-toxin in the First Fraction Precipitate which requires to be extracted with brine
<b>Diphtheria:</b>										
Lille 26/6/16	150	7.26	20	57-58° C.	8 hours	about 10 %	51 %	83.2	60	ve*
Ethel 2/5/16	350	8.62	20	"	"	negligible	50 %	90†	23	- ve (found to be 53.5 %)
Lille 26/6/16	150	7.26	saturated with NaCl	"	"	negligible	60 %	83.6	50	+ ve*
Ethel 2/5/16	350	8.62	saturated with NaCl	"	"	negligible	not tested	86.8	44	- ve (at least 20 %)*
Δ R. 35	300	6.60	10 % NaCl and 0.3 % Trikresol	"	"	negligible	50 %	89.5	66	+ ve*
<b>Tetanus:</b>										
Adelaide 1/6/16	150	6.16	8 %	"	"	negligible	50 %	85.1	70	+ ve*

\* Not tested out because of scarcity of experimental guinea-pigs.

† The heated serum was not diluted before precipitating with ammonium sulphate.



in the final product, was found to be in the First Fraction Precipitate. The phenomenon was somewhat unexpected as the percentage removal of total serum proteins was not greater than 66 and was well within the limit indicated in Table IIIa.

This observation, in conjunction with those given in Table IIIb, naturally suggested that there might be a maximum load of antitoxin which could be carried by a fixed amount of protein in the Final Product obtained by the Fractional Precipitation Methods at present in use for the concentration of sera.

In order to elucidate this point experimental concentrations of high potency antidiphtheritic sera were carried out. The sera were concentrated by Banzhaf's One Fraction Method which will yield a concentration of about 4-5 times with a removal of about 40-50 % of the total serum proteins. The same sera were also concentrated by my method which, with a heat denaturation of 30 to 40 % will yield a concentration of about 8 times.

From the results of these concentrations, which have been embodied in Table IV, it appears that *there is a limit to the amount of antitoxin which can be associated with a given weight of protein in the Final Product*: this maximum amount is of the order of 20,000 to 25,000 diphtheria antitoxin units per gramme of protein.

The possible load of tetanus antitoxin units per gramme of protein beyond 13,000 units has not been worked out as high potency tetanus antitoxic sera could not be spared at the present time for experimental work.

In those cases, Table IV, in which the load, by estimation, should have been of the order of 30,000 units per gramme of protein or more, it was found that the excess of units beyond the afore-said limit had been transferred to the First Fraction Precipitate in spite of the fact that the percentage removal of proteins was less than 80.

We thus see that, by the fractional precipitation methods at present in vogue for the concentration of antitoxic sera, the degree of concentration is limited by two factors:

- (1) The extent of the removal of the total proteins of the original serum; and
- (2) the maximum load of antitoxic units which can be associated with one gramme of protein in the final product.

There will be transference of antitoxin to the First Fraction Precipitate if the percentage removal of proteins be greater than 80 or if

TABLE IV.

*The maximum load of antitoxin which can be associated with one gramme of protein in the residues from the dialysis of the Second Fraction Precipitate (i.e. in the Final Product), in the concentration of antitoxic sera by the Fractional Precipitation Methods now in vogue.*

	Unitage of the original serum per c.c.	Protein content of the original serum %	Concentration process adopted	Denaturation induced during heating process %	Percentage of the total protein of the original serum removed during the concentration process	Anticipated unitage of the final product per c.c.	In the Final Product was found		Percentage of the total antitoxic units of the original serum appearing in the Final Product	Antitoxin carried down with the First Fraction Precipitate not extractable with 30% of ammonium sulphate but extractable with brine
							Unitage per c.c.	Protein content %		
Aurora 19/6/16	1100	10.03	Banzhaf (1913)	1.0	47	3500	3500	17.6	93	negligible
Aurora "	1100	10.03	† Homer (1916)	13.0	54.5	4500	4250	19.8	93	"
Aurora 21/8/16	900	10.03	† Homer (1916)	44.0	70	6600	4750	20.2	65	22.5 %
Aurora "	900	8.18	Banzhaf (1913)	2.0	40	3600	3000	17.2	85.5	negligible
Lloyd George 5/9/16	800	8.18	† Homer (1916)	34.5	56	5400	4750	21.3	88	"
Aurora 5/9/16	700	8.74	Homer (1916)	37.0	72	7000	4400	20.8	59	+ ve*
San 5/9/16	750	7.08	† Homer (1916)	30.0	70	6000	3600	20.6	57	+ ve*
		7.26	Homer (1916)	41.0	70	6500	4500	21.1	66.5	+ ve*

\* Owing to scarcity of experimental guinea-pigs these products were not tested out

† We have many times demonstrated that there is no appreciable loss of antitoxin during the prolonged heating of sera by my method. This was again verified with the concentrations of the sera thus indicated.

the load of antitoxin threatens to be greater than of the order of 20,000 to 25,000 units per gramme of protein<sup>1</sup>.

In a previous communication (Homer, 1916) I have drawn attention to the necessity for a study of the hydrogen ion concentration of the sera to be concentrated. Additional evidence in support of this point has been forthcoming during the progress of the present investigation. It will be seen (Table III*a*) that, in routine concentrations, the heat denaturations varied from 20 to 45 % and as a matter of practical experience it has been found that the most satisfactory end products, from the standpoint of filtration, were obtained where the heat denaturations had been of the order of 30 to 40 %.

I have ascertained that these variations are due to differences in hydrogen ion concentrations of the respective sera. Moreover, by altering the reaction of the sera it is possible to produce wide variations in the heat denaturations, although, apparently, the heat denaturation cannot be induced beyond certain limits without destruction of antitoxin.

The adjustment of the reaction of the batches of sera for concentration to a definite hydrogen ion concentration is proving of much value as it leads to uniform and consistent results. The details of the procedure adopted for the standardisation of the concentration process by an adjustment of the hydrogen ion concentration of the sera will form the subject of a further communication.

#### SUMMARY.

The following conclusions of practical value to those engaged in serum concentration can be drawn from the experimental work described above:

(1) An end product containing not more than 20 % of protein and having a potency of 8 or 10 times that of the original can be obtained as a matter of routine where the pooled antidiphtheritic sera has a unitage not greater than 500, and where the heat denaturation is of the order of 40 %.

<sup>1</sup> Banzhaf and Gibson (1907) working on an experimental scale with small quantities of unheated serum, state that, by successively fractionally precipitating with ammonium sulphate within narrow limits (50+ and 50-56 % of saturation), they have obtained a high potency fraction of the order of 45,000 units per gramme of protein.

Up to the present, working with heated sera, I have been unable to obtain a fraction with so high a unitage per gramme of protein.

(2) By the fractional precipitation methods at present employed for the concentration of antitoxic sera for therapeutic use the degree of concentration of high potency sera cannot be taken beyond the limit of about 22,500 units of antitoxin per gramme of protein in the end product.

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